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Volume XLIII, No. 5

May, 1957

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO.	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

PHYSIOLOGY

THE REPRESENTATION OF THE INTERNAL ORGANS IN THE CEREBRAL CORTEX AND CEREBELLUM OF CATS AND DOGS

COMMUNICATION I. REPRESENTATION OF THE PELVIC NERVE IN THE CEREBRAL CORTEX OF CATS

K. M. Kullanda

From the Laboratory of General Physiology (Head-Prof. V. N. Chernigovsky, Active Member of the Academy of Medical Sciences of the USSR) of the Institute of Normal and Pathological Physiology (Director-Prof. V. N. Chernigovsky, Active Member of the Academy of Medical Sciences of the USSR) of the Academy of Medical Sciences of the USSR, Moscow

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The problem of the interconnection of the cerebral cortex with the internal organs began to attract the attention of investigators in the latter third of the 19th century. One of the first pieces of work in this direction was accomplished by V. Y. Danilevsky [7], who showed in 1874 that irritation of definite portions of the cerebral cortex with an electric current produces in dogs a change in cardiac activity and in the state of the vascular system.

In the subsequent work of L. Bochefontaine [27], V. M. Bekhterev and N. A. Mislavsky [3], V. M. Bekhterev and coworkers [2], A. Cherevkov [23], B. Babkin and T. Speakman [26], B. Kaada [33] and others it was shown that in the cortex of the cerebral hemispheres of different animals a number of zones can be distinguished, the irritation of which exerts an influence on the activity of certain of the internal organs. Subsequently, work was carried out in this direction through use of the method of extirpation of different portions of the cerebral cortex and the conditioned reflex method [1, 5, 6, 10, 11, 16, 17, 20, 22 et al).

By this work and, primarily, by the numerous investigations of the academician K. M. Bykov and his school, bilateral connections were conclusively demonstrated existing between the internal organs and the cerebral cortex, and the influence of the cerebral cortex on the work of the internal organs was established.

Electrophysiological investigations occupy a chief place in the history of the problem.

Studies of the bioelectric manifestations in the brain during irritation of the nerves of the internal organs, the first of which were performed in 1891 by V. Y. Danilevsky [8], with the development of the technique of recording biocurrents led to the appearance of a number of works devoted to clarifying the influence on the cortex of irritating the internal organs and partially innervating their nerves. From the work of F. M. Lisitsa [13], E. S. Tolmasskaya [21], V. E. Delov [9], P. O. Makarov [14], F. N. Serkov [19], N. V. Bratus [4], T. E. Orlova [15] et al., it is apparent that following irritation of the internal organs diffusion changes produce the electrical activity of the cerebral cortex. In addition, in spite of the inconsistency of the data, the majority of the investigators consider that the premotor zone of the cortex has the predominant relationship to the internal organs. To put forward more definite conclusions on the basis of the facts obtained with the method indicated is not possible.

Particular attention is due the electrophysiological investigations which have been developed abroad in recent years on the primary bioelectrical reaction arising in the cerebral cortex in response to irritation of a particular nerve carrying afferent fibers from the internal organs.

By this method P. Dell and P. Olson [31] made more precise the data of P. Bailey and F. Bremer [28] relative to the cortical representation of the vagus nerve and discovered the organization of its specific and nonspecific central projections. H. D. Patton and V. E. Amassian [35] described the cortical projections of the tympanic nerve, and M. Bonvallet, P. Dell, A. Hugellin and B. Well [29] did the same for the projections of the lingual nerve. V. E. Amassian [25], C. B. B. Downman [32] and P. P. Newman [34] detected a strictly limited zone of representation in the cortex of the afferent fibers of the celiac nerve (Fig. 1).

At the suggestion of Prof. V. N. Chernigovsky we made use of the evident advantages of the method of recording the primary responses in order to collect additional material on the representation in the cerebral cortex of the afferent system of the internal organs and, primarily, to elucidate the cortical projections of the pelvic nerve.

The choice of this nerve is explained by the fact that up until the present its cortical representation has not been shown, although it is a known fact that the nerve contains a large number of afferent fibers from the organs of the lesser pelvis.

EXPERIMENTAL METHODS

The experiments were conducted on 40 cats. The animals, which were under ether narcosis, were tied to a board, after which they were injected intravenously with chloralose or Nembutal. Generally an experiment was begun under superficial anesthesia which later, depending on the purpose of the experiment, was increased to the desired extent. The central endings of the pelvic nerves were placed on submersion electrodes,* and, in a number of experiments the nerve which was on the electrodes prior to their submersion into the cavity of the lesser pelvis was perfused with neutral mineral oil at a temperature of 38° through which oxygen had previously been passed. Then a wide opening was made in the cranium, and the dura mater was removed.

In all of the experiments the shunting of the biopotentials of the cortex was unipolar. In a number of cases a localizing electrode was fastened to a manipulator which provided for a precision of up to 0.5 mm in shifting it over the surface of the cortex. The shunting was accomplished by means of cotton-paper filters soaked in Locke-Ringer's solution, tightly wound around chlorinated silver wires. In some experiments electrodes were used consisting of a silver wire suspended on a watch spring with a spheroid thickening at the end. The latter method provided the greatest certainty, inasmuch as the magnitude of the potential being shunted did not depend on a change of contact and degree of moisture of the filter.

Indifferent electrodes in the form of heavy steel needles were fastened in the bone of the cranium above the frontal sinuses. The animal was grounded and was kept warm throughout the experiment by means of a screened electric heater; the rectal temperature was recorded. In order to protect the brain cortex from chilling, it was kept warm by means of a screened heat lamp with an automatic thermoregulator and moistened with Locke-Ringer's solution heated to 38°. The experiments were conducted in an insulated room. Irritation of the central ending of the pelvic nerve was accomplished with single shocks of current from a condenser stimulator or with rectangular stimuli lasting 0.1-0.2 milliseconds from an electronic stimulator. The biopotentials of the cortex following a corresponding boost of the transformed current with capacity rheostat boosters, assembled according to a balance scheme, were conveyed to oscillographic electron-ray tubes. From the latter a photorecording was made on motion picture film. The curve of the boosters was linear within 5 to 300 cycles/sec.

EXPERIMENTAL RESULTS

The experiments showed the presence in cats in each cerebral hemisphere of two independent cortical zones for the visceral nerves, not corresponding with any of those detected earlier, in which primary responses arise during irritation of the central ending of n. pelvicus (see Fig. 1). In the present communication we shall describe the zones of the specific projections of the indicated nerve and shall not give, because of the size of the article, a detailed comparative characterization of the primary responses which were recorded in them.

* The submersion electrodes were a modified version of the electrodes suggested by P. Kiselev in the laboratory of electrophysiology of the I. P. Pavlov Institute of Physiology.

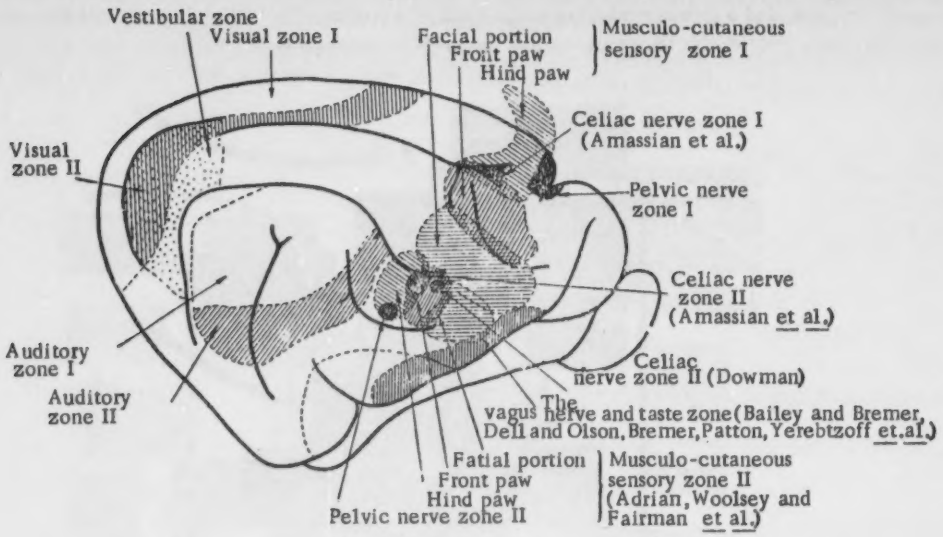
Vestibular zone
Visual zone I
Facial portion
Front paw
Hind paw
Musculo-cutaneous sensory zone I
Celiac nerve zone I (Amassian et al.)
Visual zone II
Pelvic nerve zone I
Celiac nerve zone II (Amassian et al.)
Auditory zone I
Auditory zone II
Celiac nerve zone II (Dowman)
The vagus nerve and taste zone (Bailey and Bremer, Dell and Olson, Bremer, Patton, Yerebtzoff et al.)
Facial portion
Front paw
Hind paw
Pelvic nerve zone II
Musculo-cutaneous sensory zone II (Adrian, Woolsey and Fairman et al.)

Fig. 1. Scheme of the location in the cerebral cortex of the cat of representation of different afferent systems. The map composed by P. Dell served as a basic scheme, altered and supplemented according to the data of a number of authors.

As is well known, the primary response is considered the direct bioelectric reaction of the cerebral cortex in response to irritation of receptors or afferent nerves. The principal sign characterizing this reaction is the extent of the latent period, which is subject to certain fluctuations, but which is minimal in comparison with the extent of the latent periods of the accompanying secondary reactions. In addition the primary response has a definite direction of fluctuation of potential and a characteristic duration. It is recorded only in those zones of the cortex in which are located the cells in which terminate the corresponding afferent pathways that pass through the differentiated sensory nuclei of the thalamus.*

The first of the described zones of the pelvic nerve is located on both sides of the medial and intermediate part of the sulcus cruciatus (Fig. 2). In this region the "focus of maximal activity" is located on the anterior part of the gyrus cruciatus posterior along the sulcus cruciatus, including slightly the neighboring posterior part of the gyrus cruciatus anterior. The second zone of cortical representation of the pelvic nerve is located on the gyrus ectosylvius anterior, occupying the small space along the inferior and middle part of the sulcus ectosylvius anterior (Fig. 3). In both zones when the shunting electrode is moved to the side of the focus of maximal activity the amplitude of the primary response decreases, while its latent period increases. The dimensions of the zones as well as the value of the amplitudes of the primary responses recorded in them depend on a number of circumstances. When the strength of the irritating current is augmented, the value of the amplitude of the response increases, quite rapidly attaining a maximal value; after this a further increase in current strength no longer produces an increase in amplitude. Under these conditions the boundaries of the zones of representation of the nerve under consideration are extended. With threshold values of the irritation current, the size of the zones and the amplitude of the responses depend upon the extent of narcosis, the condition of the animal and the functional state of the cerebral cortex. Increasing the narcosis leads to a reduction of the zones of representation and to a certain decrease in the amplitude of responses. Chilling the animal, low blood pressure, asphyxia, and chilling or overheating the cerebral cortex lead initially to a reduction of the zones of representation and a decrease in the amplitude value of the responses, and then to their full disappearance. In our experiments the size of the first zone was about 12 mm^2 with reasonably deep anesthesis in which the "spontaneous" activity of the cortex was depressed, while the focus of maximal activity was considerably less. Under the same conditions the area

*For detailed data on this question see A. I. Roitbak [18].

of the second zone did not exceed 8 mm². With a further increase in narcosis, for example, in the experiment the results of which appear in Fig. 3, the primary responses recorded around the focus of maximal activity (A and B) ceased to appear and a primary reaction in the form of a positive fluctuation in potential was preserved only in the spots where prior to this the responses had the greatest amplitude.

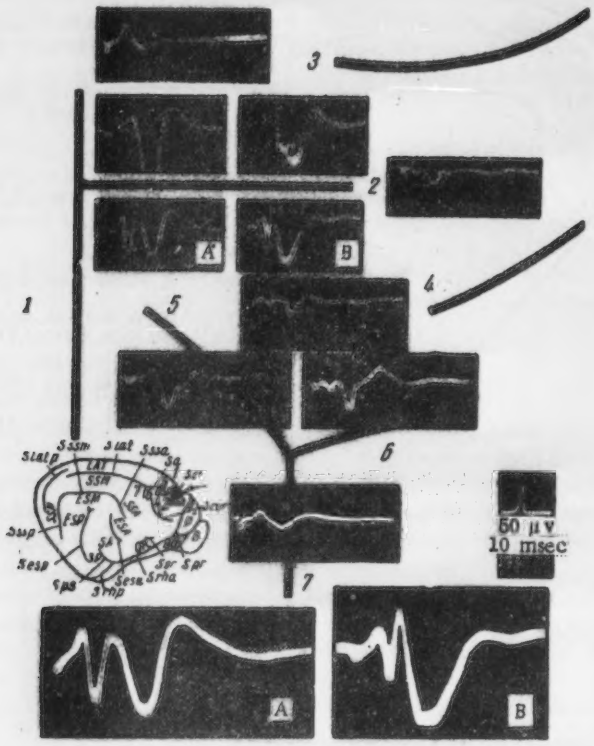

Fig. 2. Scheme of the distribution of primary responses in the right contralateral zone I of the cerebral cortex. Cat No. 85; December 9, 1955. Chloralose narcosis. Initial dose—100 mg/kg body weight. The animal was immobilized by intravenous injection of Diplacine, 0.5 cc of a 2% solution per kg of body weight. Artificial respiration. The central ending of the left pelvic nerve was irritated. A single shock of current at a voltage of 5 v served as the stimulus. The records are read from left to right. The deflection of the recorded beam downward corresponds to a positive fluctuation of potential.

1) fis. longitudinalis cer; 2) s. cruciatus; 3) s praesylvius; 4) s. coronalis; 5) s. ansatus; 6, 7) s. lateralis. On the diagram of the cerebral cortex, zone I of the cortical representation of the pelvic nerve is indicated by (↑).

When the stimulation is applied to the central ending of one of the pelvic nerves, primary responses arise in four portions of the cortex: in the two zones indicated of the contralateral (with respect to the nerve irritated) hemisphere and in the corresponding two zones of the homolateral hemisphere.

In control experiments on animals immobilized by intravenous injection of Diplacine it was established that the responses recorded do not appear as a result of possible contractions of somatic muscles. The proximity of

the second zone of the pelvic nerve to the acoustic region necessitated the elimination of audible stimuli, and this we accomplished. In a number of experiments for control purposes the pelvic neve was crushed above the applied electrodes; this completely eliminated the emergence of primary responses. The use of a cold block exerted the same effect, and after its removal primary responses were again recorded.

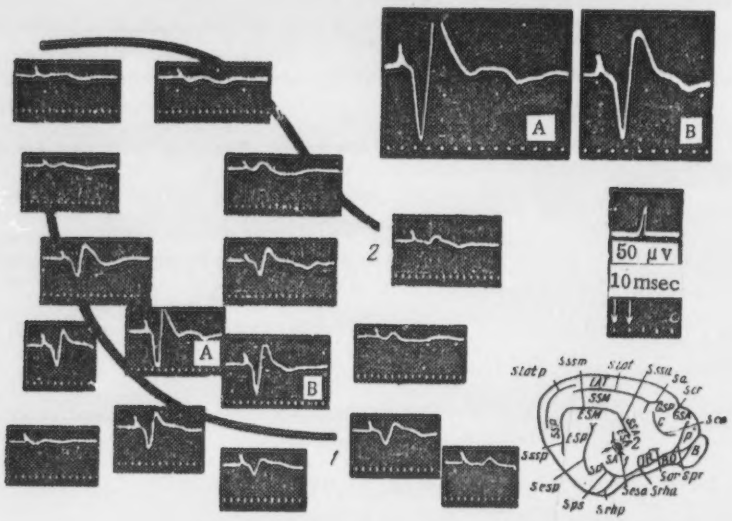

Fig. 3. Scheme of the distribution of primary responses in the right contralateral zone II of the cerebral cortex. The continuation of the experiment the beginning of which is recorded in Figure 2. The central ending of the left pelvic nerve was irritated with single shocks of current at a voltage of 4.4 v. The other conditions of conducting the experiment the same.
1) s. ectosylvius ant; 2) s. suprasylvius ant. On the diagram of the cerebral cortex, zone II of the cortical representation of the pelvic nerve is indicated by (↑), and the solid spot represents the "focus of maximal activity" (A and B).

CONCLUSIONS

The results of the work conducted afford an opportunity to consider once again a problem which still remains quite unclear—that of the structure of an internal analysor. In this question no one opinion yet prevails. Thus, for example, E. S. Airapetyants considers highly improbable the existence in the cerebral cortex of "specially defined, strictly localized portions" for an internal analysor [1], while V. N. Chernigovsky thinks that "zones of cortical representation may actually be regarded as nuclei of an analysor" [24]. While maintaining the latter opinion, we would point out that evidently the nucleus of an internal analysor has a complex structure: territorially represented in a number of zones of the cortex, within the limits of each zone it is composed of a number of "foci of maximal acitivity," corresponding to cortical projections of visceral afferent pathways.

The presence in the cortex of defined and definitely localized portions for the nucleus of an internal analysor in no measure diminishes the principle of dynamic localization of function, provided "dynamics" is taken to mean the functional interaction of processes within the limits of a single analysor (although adapted to a definite territory) and their interconnection with like processes in other analysors.

SUMMARY

Data about the localization in the cerebral cortex of cats of afferent zones of representation of the pelvic nerve are described for the first time. Single electric stimuli of the central ending of the pelvic nerve have revealed in the cerebral cortex two zones registering primary responses of maximum amplitude and minimum latent period.

Zone I is localized on both sides of the medial and intermediate parts of s. cruciatus. Zone II is situated on g. ectosylvius ant., occupying a small space along the lower and middle parts of s. ectosylvius ant. The area of Zone I is about 12 mm^2 and Zone II about 8 mm^2.

Both of these areas are bilateral, and stimulation of the pelvic nerve elicits a primary reaction in four portions of the cortex: in contralateral Zones I and II and in the corresponding ipsilateral Zones I and II.

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ABSOLUTE AND RELATIVE DURATION OF VENTRICULAR SYSTOLIC PHASES

V. L. Karpman

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The present communication includes data on the duration of the phases of isometric contraction and expulsion of blood from the ventricles obtained from examination of 100 healthy persons. As a method which would permit a phase analysis of the cardiac cycle we chose the dynamocardiograph proposed by E. B. Babsky and co-workers [1].

The average duration of the fundamental systolic phases of the ventricle and the limits of the variations in the length of these phases under normal conditions have been adequately studied at the present time. Individual variations in ventricular systolic phases are determined principally by the heart rate. It is well known that with increased frequency of heartbeat there is a reduction in the length of ventricular systole [6] and in the length of the blood expulsion phase [8]. There were no indications in prior literature of a relationship between the duration of the isometric contraction phase and heart rate [4]; in 1953, however, Askanas [3] noted this relationship.

Data are listed in the table which demonstrate at a glance the relationship between the duration of both phases of ventricular systole and heart rate.

This relationship is so precise that, as will be shown below, it can be expressed mathematically.

As well as examining the absolute duration of ventricular systolic phases, we are also studying their relative length. We call the relative length of any ventricular systolic phase the ratio, expressed in per cent, of the absolute duration of the given phase to the total length of the systole as a whole. Since the relative length of the isometric contraction phase and the expulsion phase together total 100%, for judging intrasystolic relationships an analysis of any one relative value is completely adequate. We utilize the relative length of the blood ejection phase, which we call the intrasystolic index (ISI):

$$ISI = \frac{\text{length of the ejection phase in seconds } (t_e)}{\text{length of ventricular systole in seconds } (S)} \times 100\% \qquad (1)$$

From this expression it is apparent that the intrasystolic index is a relative value which shows what part of the entire time of contraction of the ventricles is taken up by the expulsion of blood from them into the main vessels.

The values of the ISI computed for healthy individuals of different sex and age fluctuate within comparatively narrow limits—from 69 to 81%, with an average value of 75%. Thus the explusion of blood from the

ventricles lasts approximately $^3/_4$ of systole, and consequently their isometric contraction amounts to only $^1/_4$ of systole.

Low values of ISI (69-72%) are encountered among people over 40 years of age, while high values (78-81%) are found among young individuals who are physically well developed. When muscles are doing work, the value of the ISI value increases in comparison with its value during the resting state. With certain heart ailments (mitral stenosis, myocardial infarct, etc.) the value of the ISI is considerably lower than under normal conditions. This indicates that value of the ISI depends on the functional state of the heart muscle; and therefore the given index can be used to evaluate the effectiveness of cardiac contraction.

The relative duration of the phases of systole, in opposition to their absolute duration, does not depend on the heart rate (see table). This means that with increased or decreased frequency of heartbeat, in which there occurs respectively a shortening or a lengthening of ventricular systole as a whole, the relationship between the

TABLE

Duration of the cardial cycle C (in seconds)		Duration of the expulsion phase t_e (in seconds)			Duration of the isometric contraction phase t_t in seconds			Intrasystolic index (average experimental data (in %)
Variation limits	Average duration	Average experimental data	Computed according to the formulae		Average experimental data	Computed according to the formulae		
			$t_e = 0{,}211\sqrt{C}$	$t_e = 0{,}137 \cdot C + 0{,}106$		$t_t = 0{,}081\sqrt{C}$	$t_t = 0{,}046 \cdot C + 0{,}035$	
0.60—0.69	0.628	0.192	0.192	0.192	0.065	0.064	0.064	74.7
0.70—0.79	0.735	0.207	0.208	0.207	0.069	0.069	0.069	75.0
0.80—0.89	0.828	0.222	0.222	0.219	0.072	0.073	0.073	75.5
0.90—0.99	0.927	0.231	0.234	0.232	0.075	0.077	0.077	75.5
1.00—1.09	1.025	0.244	0.245	0.246	0.079	0.081	0.082	75.5

individual phases is not altered. Hence it follows that both with a rapid and with a slow heart rate the time of expulsion of blood from the heart into the vessels continues to take up $^3/_4$ of the time of systole as a whole, and the time expended in creating the pressure necessary to open the semilunar valves still amounts to $^1/_4$ of systole. This fact seems extremely important to us, because it characterizes the definite regularity of heart contraction in the presence of physiological variations in heart rate.

The independence of the ISI from heart rate makes considerably easier the mathematical analysis of the relationship between the length of the phases of systole and the frequency of heart contractions. Thus, substituting in expression (1) the formula for the length of ventricular contraction with varying heart rate, $S = 0.324\sqrt{C}$ (2), and transcribing it with respect to t_e, we obtain after simple conversions a formula characterizing the dependence of the time of expulsion of blood from the ventricles on heart rate:

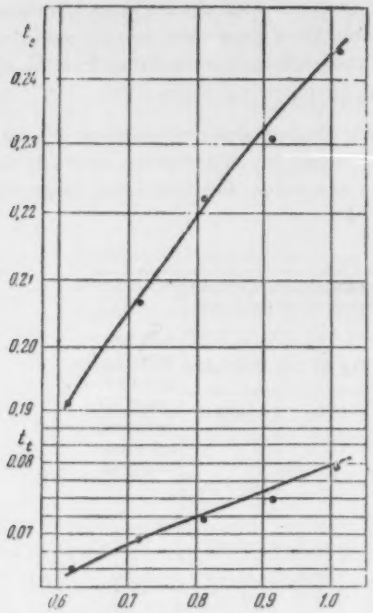
$$t_e = 0.244\ \sqrt{C}\ , \qquad (2)$$

where C is the duration of the cardiac cycle in seconds.

Proceeding from the fact that the relative length of the isometric contraction phase equals 0.25 or 25%, its absolute length (t_t) will be three times shorter than the absolute length of the ejection phase. Hence the relationship between the duration of the isometric contraction phase and heart rate can be written in the following form:

$$t_t = 0.081\ \sqrt{C} \qquad (3)$$

As is apparent from the table and the graph, the values of the length of the ventricular systolic phases computed according to formulas (2) and (3) and their values obtained by analysis of dynamocardiograms are extremely close.



The relationship of the duration of the isometric contraction phase of the ventricles (t_t) and the phase of expulsion of blood from them (t_e) to the duration of the cardiac cycle (C). The lines are constructed according to formulas (2) and (3); the experimental points are derived from the table.

When it is taken into account that the curve of the functions is negligible in the studied range of variation of the independent variable, the relationship between the duration of the isometric contraction phase, the expulsion phase and the heart rate can be expressed in a type of straight line equation.

For this, repeating the reasoning adduced above with the single difference that the value of S is equal to $0.183 \cdot C + 0.142$ [8], we obtain the following expressions:

$$t_e = 0.137 \cdot C + 0.106 \qquad (2)$$

$$t_t = 0.046 \cdot C + 0.035 \qquad (3)$$

As is evident from the table, these formulas also give values close to the experimental values.

According to data in the literature which is known to us, the mathematical relationship between the duration of the phases of systole and heart rate has been noted only for the phase of the expulsion of the blood from the ventricles [5]. This function, however,

$$t_e = a \sqrt[2.9]{p}$$

is inconvenient in practical work, since in order to determine the duration of the ejection of blood with a given heart rate prior use of logarithms is required.

From a comparison of our formula, $t_e = 0.244\sqrt{C}$, with the formula characterizing the relationship between the duration of the interval H–K of a ballistocardiogram and heart rate, $H–K = 0.247\sqrt{C}$ [6], their close concurrence is evident.

Apparently the H–K interval of a ballistocardiogram, which is called "hemodynamic systole," is a reflection of the phase of expulsion of blood from the ventricles

SUMMARY

The duration of the isometric contraction and of the ejection of blood from the ventricles may depend on heart rate. The dependence of isometric contraction (t_t) and blood ejection (t_e) can be expressed by the formulas:

$$t_e = 0.244 \cdot \sqrt{C} \text{ and } t_e = 0.137 \cdot C + 0.106$$

$$t_t = 0.081 \cdot \sqrt{C} \text{ and } t_t = 0.046 \cdot C + 0.035$$

The idea of an intrasystolic index is adopted: it is the relation between the duration of the ejection phase and the duration of ventricular systole. This index estimates the efficiency of heart contraction. The relative length of the ejection phase (intrasystolic index) and that of the isometric contraction phase are practically independent of heart rate.

length of the ejection phase (intrasystolic index) and that of the isometric contraction phase are practically independent of heart rate.

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IONO-HUMORAL INTERRELATIONS DURING THE APPEARANCE AND DEVELOPMENT OF VAGAL INHIBITION OF THE HEART

COMMUNICATION III. CHANGES IN THE EFFECT OF THE VAGUS NERVE WITH ATROPINIZATION OF THE HEART

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(Received December 29, 1954. Presented by Prof. M. S. Vovsi, Active Member of the Academy of Medical Sciences of the USSR)

In spite of the fact that data exist in the literature on the change in the effect of stimulating the vagus nerve on atropinized visceral organs, until now the idea of the exclusion of the effect of the vagus nerve by means of atropine has been widespread in physiology.

In Communication I we set forth experimental data on the effect of atropine on a mechanogram and electrogram of the heart. It was established that atropine increases and stabilizes the resting potential of the heart muscle, and does not alter the form and duration of the monophase action potentials. The data of the report showed a change in the effect of acetylcholine on an atropinized heart; under these conditions acetylcholine proved to be incapable of changing in the usual way the rate of the restored processes taking place in the heart following depolariziation of cellular structures. These data permitted us to suggest that atropine, stabilizing the resting potential of the heart muscle, must first of all disturb the normal process of an increase in polarizibility of cellular structures during stimulation of the vagus nerve. Proceeding from the experimental data and the theoretical conclusions of M. G. Udelnov [2, 3], we assumed that under these conditions the greatest changes will be observed in the chronotropic index of vagal inhibition of the heart.

The present communication is devoted to an examination of the forms of the manifestation of the effect of the vagus nerve on an atropinized heart.

EXPERIMENTAL METHODS

The experiments were conducted during all seasons of the year on frog hearts isolated according to A. F. Samoilov's method. The use of the A. F. Samoilov preparation (the heart on a Straub cannula and in a circular perfusion system, connected with the frog's head by the vagosympathetic trunks) permitted stimulation of the nucleus of the vagus nerve in the medulla oblongata and the cephalic sympathetic ganglion. This method made it possible to obtain pure vagus and sympathetic effects during parallel perfusion of the isolated heart. Stimulation was accomplished by means of movable needle electrodes joined with a Dubois-Reymond coil; the voltage in the primary circuit was 4 v. Cardiac mechanograms and electrograms maintained simultaneous recording (see Communication I).

Following the recording of 3-5 normal vagal effects on a heart perfused with Ringer solution, atropine was introduced into the cannula, and at varied time intervals repeated stimuli were applied to the medulla oblongata.

EXPERIMENTAL RESULTS

In all of the experiments, following atropinization of the heart the effect of vagal inhibition was replaced by a marked sympatheticomimetic effect. The change in vagal effect after the injection of atropine into the heart in a concentration of 10^{-5} can be seen in Figure 1 (I and II). The sympatheticomimetic effect is manifested in an increase in the strength of contractions and in the speed of the rhythmic activity of the heart. This effect is highly stable; in the experiment cited, 14 subsequent stimulations gave identical results (Figure 1, II). After 40 minutes the atropine solution was replaced with Ringer's solution and stimulation of the medulla oblongata was once again accompanied by an inhibitory effect (Figure 1, III).

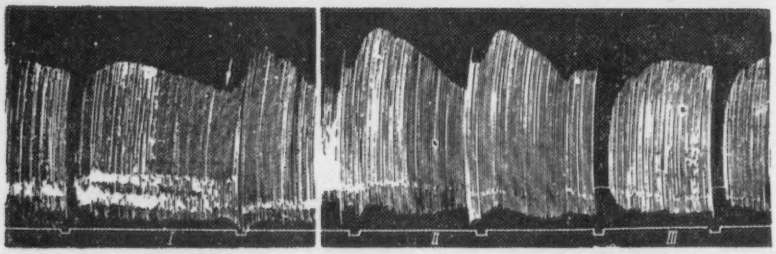

Fig. 1. The change in vagal effect under the influence of atropine (10^{-5}). Vagal effect in conjunction with stimulation of the medulla oblongata before and immediately after the action of atropine (I), 1 hour after the action of atropine and following washing out with Ringer's solution (II). Distance between inductor coils —12 cm. Duration of stimulation —10 seconds.

During the course of the experiments we were faced with the question of the nature of the sympatheticomimetic effect. In Figure 2, a, Segment I shows the typical inhibitory effect of stimulation of the medulla oblongata; a small increase in the amplitude and strength of the contractions of the heart is apparent in the aftereffect. In spite of differential stimulation of the fibers of the vagus nerve, one could assume that after the introduction of atropine precisely this sympathetic aftereffect is stengthened. Segments II and III show the development of a stable sympatheticomimetic effect during stimulation of the medulla oblongata after the introduction of atropine (in the given experiment 12 identical results were obtained). Figure 2, b shows the change in the effect of stimulation of the cephalic sympathetic ganglion after atropinization of tne heart: the introduction of atropine reduces the sympathetic effect (this phenomenon was observed in all of the experiments). Figure 2,c completely eliminates the possibility that the participation of sympathetic fibers in the development of sympatheticomimetic effects is a result of stimulation of the vagus nerve. This kymogram was obtained on a permanently desympathetized frog heart; even in this case stimulation of the medulla oblongata after the introduction of atropine was accompanied by a marked sympatheticomimetic effect.

In studying the relationships of the inotropic and chronotropic properties of vagal inhibition during atropinization of the heart, we established that stimulation of the medulla oblongata immediately after injection of atropine in concentrations of $10^{-6} - 10^{-5}$ is accompanied by the development of a positive chronotropic effect; the negative inotropic effect disappears after a certain period of atropine influence. Thus atropine affects first of all the chronotropic index of vagal inhibition. This regularity was particularly well-marked in the experiments with continuous perfusion of the heart.

This continuous perfusion is effected by a Straub cannula with a side outlet; introduced into the cannula is a small funnel with a fine, almost capillary outlet which passes freely into the horn of the cannula. The small funnel is filled with Ringer's solution, which is continuously renewed by means of drops falling from a large funnel fastened above the cannula; the excess fluid exits from the side outlet of the cannula. If an active substance (atropine, for example) is introduced into the small funnel, it will at once begin to be washed out by new drops of Ringer's solution. In this manner one can observe the change in vagal effect as atropine is washed out of the heart.

The experiments showed that with continuous perfusion the negative inotropic effect is restored first of all (in the presence of a positive chronotropic effect), and then a negative chronotropic effect is produced by stimulation of the medulla oblongata.

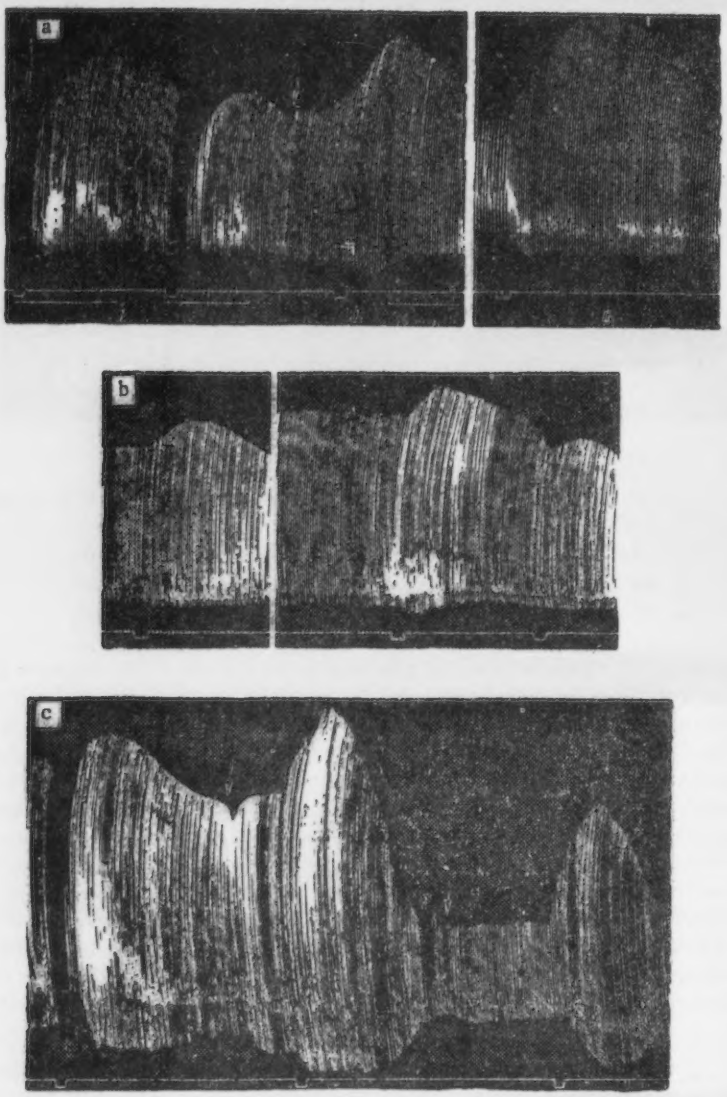

Fig. 2. Change in vagal effect in conjunction with stimulation of the medulla oblongata (a) and the sympathetic ganglion (b) on a normal and a permanently desympathetized heart (c) before and after (↓) the action of atropine (10^{-5}). Distance between the inductor coils in the experiments on the normal heart (a, b) –19 cm; in the desympathetized heart (c) –12 cm. Duration of stimulation –10 seconds.

In this connection it was necessary to establish the sequence of changes in the reaction of the hearts to acetylcholine and stimulation of the vagus nerve during atropinization of the heart and washing it with Ringer's solution. The experiments showed that after atropinization the capacity of cardiac muscle to respond to stimulation of the medulla oblongata is initially altered by a reduction in heart rate; then the negative inotropic effect

disappears. Finally the reaction of the heart to acetylcholine is changed.

The literature contains indications of the great sensitivity of the chronotropic effect to atropine [5, 6].

Thus one can conclude that in the process of atropinization of the heart exclusion of the vagal effect does not occur, but only the form of its manifestation is altered.

Stimulation of the vagus nerve under these conditions is accompanied by positive chronotropic and inotropic effects, which increase in the process of gradual atropinization, attain a maximal value and are stablized. It has been established that sympathetic fibers do not take part in this reaction; the inaccuracy of the assumption of the possibility of the stimulation of chromaffin tissue is shown in the work of A. V. Kibyakov and L. V. Tukhvatullina [1].

In our supplementary experiments the atropinized heart secreted a greater than normal amount of vagal substance. These data are in agreement with the results of Brucke [4]. Consequently the sympatheticomimetic effect does not result from the secretion and influence of small amounts of acetylcholine.

This effect cannot be caused by the activiation of accelerating fibers in the vagus nerve trunk, since in the work of M. G. Udelnov [2, 3], the absence of specific accelerating fibers in the trunk of the vagus nerve of the frog and of warm-blooded animals has been demonstrated.

In view of the particular sensitivity of the chronotropic effect of vagus inhibition to the action of atropine, we examined the shifts in polarization which occur in an atropinized heart in conjunction with stimulation of the vagus nerve.

In our theoretical assumptions we proceeded from the data of M. G. Udelnov [2, 3], who established a causal relationship between reduced heart rate and increased resting potential in conjunction with stimulation of parasympathetic nerves.

The vagal effect was produced either by placing a small crystal of rock salt on the nuclei of the medulla oblongata or by their electrical stimulation. Figure 3 shows the development of the vagal effect before and after atropinization. As is evident from Figure 3, a, placing a small salt crystal on the medulla oblongata before

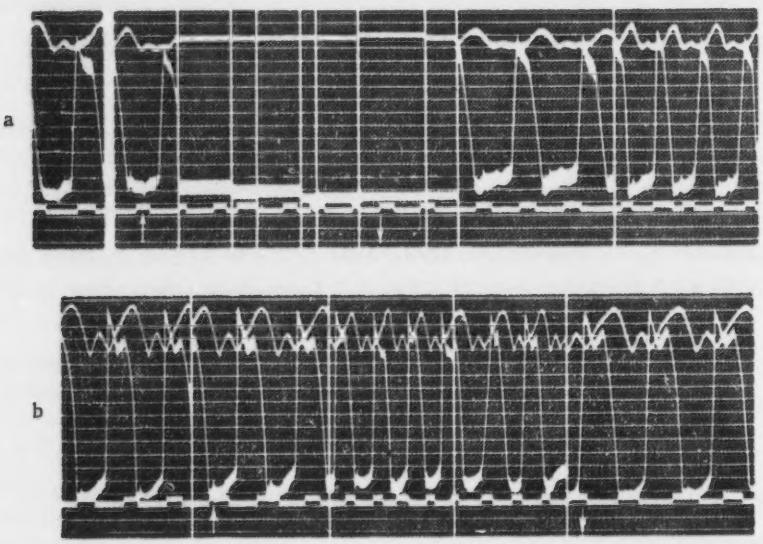

Fig. 3. Mechanograms (upper curves) and electrograms (lower curves) of the heart, recorded in conjunction with stimulation of the medulla oblongata with a small crystal of NaCl.

atropinization is accompanied by the development of cessation of the heart and an increase in the resting potential of the cardiac muscle. Following atropinization (Fig. 3, b) during stimulation of the nuclei of the vagus nerve a slight increase in heart rate is observed in the presence of a parallel reduction in resting potential and a lowering of the amplitude of the monophase action potential. A similar phenomenon was recorded in all 65 cases.

Thus the electrophysiological experiments also attest to a change in vagal effect, but not its exclusion, under the influence of atropine.

The results obtained in comparison with the data in the literature permit one to assume that atropine prevents the possibility of positivization of cardiac muscle in the presence of stimulation of the vagus nerve. Atropine by itself is capable of augmenting the resting potential, but, collaterally, it emerges also as a stabilizer of the level of polarity of the cardiac muscle. Accepting the point of view of M. G. Udelnov, one can maintain that the number of impulses arriving by way of the vagus nerve is insufficient in the atropinized state for the creation of positivization, and thus for inhibition of the heart. Under these conditions the usually "inhibitory" impulsation produces an effect of negativization, characteristic of the action of a sympathetic nerve; there occurs a parallel increase in heart rate and in the amplitude of heart contractions. In this case the minor amount of impulses arriving by way of a sympathetic nerve is incapable of producing any effect.

We suggest that the data presented confirm the viewpoint of M. G. Udelnov relative to the leading role of shifts in polarization during the emergence and development of vagal inhibition of theheart. They also show that atropine influences first of all the initial resting potential of cardiac muscle, eliminating the possibility of changing it during development of vagal inhibition of the heart. The absence of positivization under these conditions causes the replacement of the inhibitory effect from irritation of the vagus nerve by one of stimulation.

SUMMARY

In normal atropinized and chronically desympathetized frog hearts the effect of vagus nerve stimulation is altered but not excluded.

Stimulation of the nuclei of the vagus nerve in the medulla oblongata causes a constant sympatheticomimetric effect. The same is to be seen in chronically desympathetized frogs. During atropinization and washing the negative chronotropic effect caused by vagus stimulation was replaced by a positive one. The last to be altered was the reaction of the heart to acetylcholine.

The atropinized heart secretes more vagus substances than the normal one.

Stimulation of the vagus nuclei after atropinization causes a slight diminution of the resting potential of the heart muscles, diminishing at the same time the amplitude of monophase action potentials.

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AN INTERPRETATION OF CERTAIN PECULIARITIES OF THE ARTERIAL OSCILLOGRAM OF CHILDREN*

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One of the most marked characteristics of children's arterial oscillograms is the magnitude of vascular fluctuations, which is less than that found among adults. The oscillographic index is also smaller.

The results of processing 359 oscillograms, which we recorded among 164 individuals 12 to 25 years of age in a state of rest, show that a small magnitude of oscillations (up to 10 mm) until the age of 16 is encountered in more than to 50% of cases, while among adults it is encountered in only 4%. High oscillograms (over 20 mm) prior to 16 years of age are rarely encountered; among adults they are found in more than half of the cases.

The oscillographic index (OI) changes not only with age, but also under the influence of physical stress. The physiological interpretation of the changes resulting from athletic training, the stress of training persuits, presents considerable difficulties.

No definite opinion is to be found in the literature relative to the causes of the change in OI. In the opinion of some authors [15, 18] the oscillographic index characterizes predominantly the work of the heart. Others consider the oscillographic index to be an indication of the tonus of the arterial wall. A third group suggests that the oscillographic index depends to the same degree both on the work of the heart and on the state of the arterial wall [12, 14, 22].

The conclusions drawn by the author were made on the basis of clinical observations, model experiments (with rubber tubing) or the results of the use of vasoconstricting and vasodilating substances. The amount of experimental work in this area in comparison with the number of clinical observations is very slight.

The aim of the present work is to establish whether it is possible by the change in a young individual's OI after physical exertion to judge the change in functional state of the heart.

EXPERIMENTAL METHODS AND RESULTS

We studied the relationship of the OI, determined by means of a Serkin-Kudenko arterial oscillograph, and the diastolic-systolic difference in heart area calculated by the planimetric method in conformance with a roentgenokymogram (RKG) in direct projection. We did not succeed in finding similar examinations in the literature.

* Presented at the Post-graduate Conference of the Central Scientific Research Institute of Physical Culture, May 12, 1956.

The planimetric method of computing heart area in diastole and systole according to an RKG makes it possible to judge the contractile capcity of the heart [1] and the magnitude of its pulsatile volume [2, 5, 8].

Eighteen young individuals were examined: 12 aged 12-14 and 6 aged 15-16. All of the youths were healthy and were engaged in light athletics in a juvenile school of sports.

Oscillograms and RKGs were made simultaneously, at rest and 20 seconds after 40 deep knee-bends.

No clear-cut relationship was found between the magnitude of the OI and the diastolic-systolic difference in heart area in the state of muscular rest; as is shown by the data listed below (Table 1), along with the same value of OI both small and large values of the diastolic-systolic difference in heart area were observed.

TABLE 1

Values of the Oscillographic Index and Diastolic-Systolic Difference in Heart Areas at Rest

Number of observations	Value of oscillographic index (in mm)	Value of diastolic-systolic difference in heart areas (in cm^2)
1	20	10
2	17	13
3	16	12
4	14	17
5	14	12
6	12	12
7	12	3
8	11	9
9	11	8
10	10	10
11	8	6
12	8	7
13	8	11
14	8	7
15	7	9
16	7	4
17	7	13
18	6	11

The absence of a relationship between the changes in these two values was especially clearly evident after physical exertion (Figure 1). Among the majority the oscillographic index increased after exertion, while the

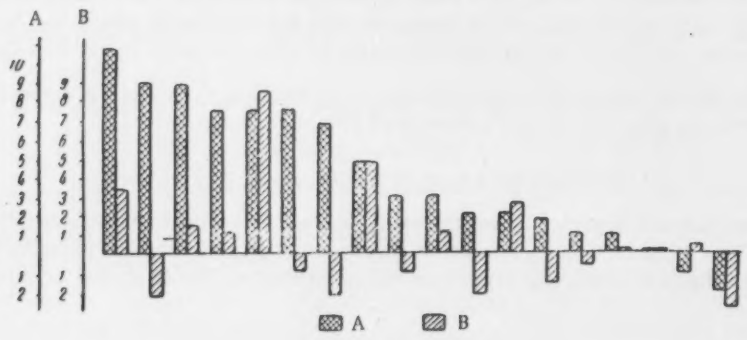

Fig. 1. Change in oscillographic index in mm (A) and in diastolic-systolic difference in heart areas in cm^2 (B) after 40 deep knee-bends. The zero line is the resting level. The columns located below the zero line designate a decrease in the indices. The two contiguous columns represent changes in the same individual.

difference in diastolic-systolic heart areas was reduced in almost 50% of the cases. Thus one cannot judge the work of the heart by the change in oscillographic index.

The large values of pulsatile volume of blood [13, 20, 21], as well as the comparatively large heart sizes among youths [1, 4, 6], indirectly confirm our conclusion.

A comparison of the values of the oscillographic index with the other characteristics of an oscillogram furnishes an identical trend in their changes.

After comparing the changes in oscillographic index following 40 deep knee-bends with the changes of the third phase (the right portion of the oscillogram from the largest peak to a sudden fall-off of oscillations according to M. V. Kudenko), and with the change in length of the entire oscillogram, it is evident that the direction of the changes of the third phase (Figure 2) and of the length of the oscillogram (Figure 3) in the majority of cases corresponds with the direction of the change in oscillographic index. Lack of correspondence is observed in the case of extreme changes in oscillographic index, both of the largest and the smallest changes.

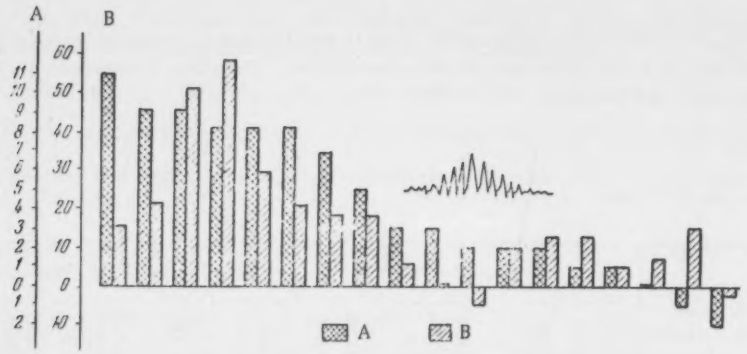

Fig. 2. Change in oscillographic index in mm (A) and in the third phase of the oscillogram in mm of mercury (B) after 40 deep knee-bends. The other designations are the same as in Figure 1.

It was assumed that the third phase and the length of the oscillogram are indications of vascular tonus [7, 18]. A comparison of the changes in oscillographic index, length of the oscillogram and the third phase provides grounds for the assumption that the change in the magnitude of OI after physical exertion is determined to a large extent by the state of the arterial wall.

Many investigators [3, 17, 19] consider a small oscillographic index among adults to be a sign of increased vascular tonus. In a young person, to all appearances, this is not the case. According to our data, the short length of the third phase and of the entire oscillographic curve, as well as a small value of A. S. Lebedev's index (the difference between maximal pressure values, determined by oscillographic and ausculatory methods [10, 11]) are rather an indication of reduced arterial tonus at this age. Our data on the value of A. S. Lebedev's index at different ages are presented in Table 2.

Thus the number of cases with a positive value of A. S. Lebedev's index grows with age, increasing suddenly toward 16 years of age. A zero value of this index is encountered in almost 50% of all cases between ages 12 to 14. Negative values of A. S. Lebedev's index are not encountered at all above age 16.

Simultaneous use of the methods of arterial oscillography and RKG made it possible to obtain new data which indirectly attest to the presence of complex neuro-humoral regulation of the vascular system. If the latter were a simple physical system of tubes, then with an increase in the pulse wave characterizing the work of the heart there would be an increase in the stretching of the vascular wall, its fluctuations and, consequently, in the oscillographic index. This, however, does not occur, inasmuch as, to all appearances, each change in

the work of the heart reflexly (chiefly through the system of pressoreceptors of the sinocarotid zone) produces a corresponding change in vascular tonus, a fact which makes it impossible to judge the state of the heart by the oscillographic index.

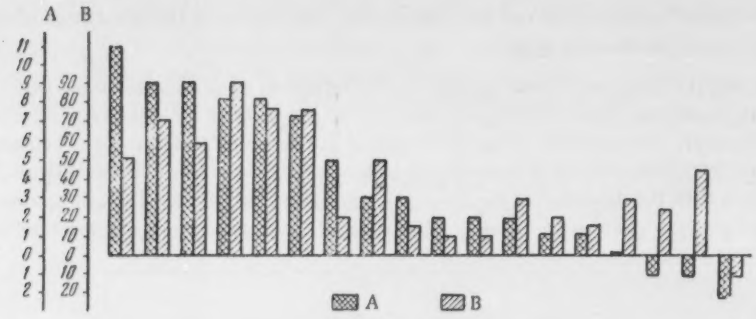

Fig. 3. Change in oscillographic index in mm (A) and in length of oscillogram in mm of mercury (B) after 40 deep knee-bends. The other designations are the same as in Figure 1.

TABLE 2

A. S. Lebedev's Index in Relation to Age

Value of Lebedev's Index	12 yrs.		13 yrs.		14 yrs.		15 yrs.		16 yrs.		17 yrs.		18 yrs.		20–25 years	
	Number of cases	in %	Number of cases	in %	Number of cases	in %	Number of cases	in %	Number of cases	in %	Number of cases	in %	Number of cases	in %	Number of cases	in %
Negative	4	12	9	15	7	8	5	20	—	—	—	—	—	—	—	—
Zero	17	51	25	40	38	45	6	24	1	7	1	9	1	10	7	23
Positive	12	37	27	45	40	47	14	56	13	93	10	91	8	90	19	77
Total number of cases	33		61		85		25		14		11		9		26	

Consequently the small OI typical among juveniles does not indicate slight contractile capacity of the heart or a small pulsatile blood volume at this age, either at rest or after physical exertion.

SUMMARY

Simultaneously recorded values of oscillographic index (O. I.) and diastole-systole difference in heart area were determined planimetrically on kymograms in the state of muscle relaxation and after physical change in different directions. This leads us to assume that: 1) change of O. I. does not adequately show functional conditions of the heart in connection with physical exertion; 2) low O. I. in juveniles does not necessarily imply insufficient functioning of the heart; 3) lack of correspondence in the change of O. I.-values and of diastole-systole difference in heart area reveals complex relations in the realm of neurohumoral regulation of blood circulation.

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A COMPARISON OF CONDITIONED REFLEXES USING DIFFERENT REINFORCING STIMULI

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In determining types of higher nervous activity, the standard tests are used, and in these the essential indications are given by conditioned reflexes based on response to food. However it is being reported with increasing frequency in the literature, that the conditioned reflex activity of one single animal, determined on the basis of only one kind of reinforcement, may differ from that determined by using a different reinforcing stimulus.

I. I. Korotkin [5], using feeding and defense as reinforcements, found that only in 2 out of the 9 children investigated was there agreement in respect of type, when two different reinforcements were used.

O. P. Kapustnik and V. K. Faleeva [3] report a different rate of extinction of conditioned reflexes in children, according to whether the reinforcement was by feeding, orientation, or speech.

L. E. Khozak [11], in establishing conditioned reflexes and inhibitions using food, orientation, and defensive reaction, as reinforcements in children, found that according to the three methods, one child could be classified as belonging at the same time to several different higher nervous activity types.

A. G. Ivanov-Smolensky [2], dealing with work of this kind, thinks that "together with the general sympathetic type of higher nervous activity, one may also talk of a partial, component type, whose characteristic is either a heightened or a lowered feeding, defense, sexual, or exploratory activity, or a greater or less tendency to establish conditioned associations." He considers this to apply not only to increase or decrease in excitability, but also to lability or inertia.

A. P. Chesnokova [12], M. S. Alekseev [1] and M. V. Troitskaya [9], working with animals, using food and acid as reinforcements, and applying a low standard in defining higher nervous activity, found different characteristic types according to the reinforcement used.

It can be seen from the literature, therefore, that one cannot determine the type of higher nervous activity of an animal, purely on the basis of the feeding method.

In the present paper we report results obtained during an investigations of the relation between conditioned and unconditioned reflexes, in the case of reinforcement with food, acid, or electrical stimulation of the skin. In this way, we can compare the conditioned reflex responses obtained with each kind of reinforcement in one and the same animal, i.e., we can describe the characteristics of a type in a greater number of ways.

It must be noted that we made a more or less detailed determination of type, only in the case feeding reinforcement; when using acid or giving electrical skin stimuli, we investigated only the rate of development, magnitude, and consistency of the conditioned reflexes, and the speed and stability of the differentiation. Taking into account that all these indications are of considerable importance in determining an animal type, we can now to some extent determine a type of higher nervous activity on the basis of experiments using several different reinforcing stimuli.

In the case of conditioned reflexes to food or acid or to stimulation of the defense reaction, we had to break up the stereotyped behavior and to reinforce the differentiated stimulus, and this allowed us to form conclusions as to what type the animal was when acid was given or electrical stimuli were applied to the skin. When we compared the different indices of conditioned reflex activity in each animal, obtained by using different reinforcements, we found that conditioned reflex behavior with one kind of reinforcement was sharply distinguished from that shown in response to another kind, and that the strength of the processes in the cortex might be quite different according to the kind of unconditioned stimulus.

The results are shown in the following table.

Conditioned Reflex Behavior with Different Kinds of Reinforcement

Dogs used	Reinforcement with		
	Food	Acid	Electrical stimulation of skin
Irtysh	Strong balanced (Sanguine)	Strong unbalanced	Strong balanced
Dozor	Strong balanced (phlegmatic)	Weak	Strong balanced
Ruslan	Strong unbalanced	Weak	Weak
Agap	Weak	Strong balanced	Weak
Golub	Weak	Strong unbalanced	Strong unbalanced
Boi	Weak	Weak	Weak

From this table we see that of the 6 dogs used in the experiment, in 2 there was agreement as to type for all three reinforcing stimuli; one of these was shown in each case to have the strong type of nervous system, and the other the weak type in all three tests. In the remainder, the conditioned reflex behavior was a function of the kind of reinforcement. We must however point out once more that in talking of the defense reflexes, and those stimulated by acid, we refer only to the reflex in a general way and have not carried out the special tests required for an accurate determination of the type of higher nervous activity.

It is known that the subcortical centers constitute a source of energy which is necessary for the activity of the cortical cells. It is also known that the different conditioned reflexes are of different strengths in the one animal. It is natural therefore, when the reinforcement is related to a strong unconditioned reflex, that the unconditioned reflex behavior will be associated with strong cortical processes, and conversely, when the reinforcement affects only a weak unconditioned reflex, the conditioned reflex activity will be characterized by the weakness of the cortical processes.

We must also emphasize that in nearly all the animals, with reinforcement by acid or by electrical stimulation of the skin, differentiation was obtained with far greater difficulty than when food was used.

The explanation of this is to be found in the tendency, in the case of the defense reflexes, for the excitatory condition to radiate, a tendency which results in a greater degree of generalization of the positive conditioned reflex.

SUMMARY

In the course of establishing conditioned reflexes in dogs on the basis of unconditioned – food, acid and motor – defensive reflexes, typological peculiarities of each dog have been studied. Conditioned reflex activity under all of the three reinforcements has shown a coincidence of typological peculiarities in two dogs – one of the strong, the other of the weak type of nervous activity. As to the remaining four dogs each demonstrated individual typological peculiarities to each reinforcement of conditioned reflex activity. In almost all of the dogs the inhibitory cortical process was relatively weaker than the stimulating one.

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ACCOMMODATION IN PERIPHERAL MOTOR NEURONS DUE TO PROPRIOCEPTOR IMPULSES

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The question of the effect of proprioceptor stimulation on central neurons is one which has long attracted attention. The aim of the present investigation is to throw light on the part played by the proprioceptor impulses in the accommodative process, by observing the rate of accommodation in peripheral motor nerve fibers.

METHOD

The experiments were performed on the frog Rana esculenta in autumn and winter. The brain was removed one hour before the experiment, and a preparation was made of the sciatic plexus and the muscles appropriate to the particular experiment. One of these muscles was connected to a myograph, to record the contractions.

For stimulation of the sciatic plexus we used a slightly altered version of Solandt's circuit, giving either a square wave or an exponentially rising wave form; by means of a two-way switch, either of these wave forms at various strengths could be applied to the sciatic plexus. Nonpolarizing electrodes were used.

The accommodation of the motor nerve was determined graphically by recording side by side the contractions evoked by stimulation of the sciatic plexus with a square and with an exponential wave form, and this also served as an index of the rate of change of accommodation.

RESULTS AND DISCUSSION

From the beginning we were convinced that the proprioceptor impulses have an effect on the accommodative power of the motor nerve, through changes in the relations in the nerves at nerve centers. One gastrocnemius muscle was stretched with a load of 50-100 g; the sciatic plexus of the opposite side was stimulated by applying either square wave or exponentially rising voltages, and the resulting muscle contractions were recorded; in this way both increases and decreases in the accommodative power of the motor neuron were observed. A two phase change in the accommodative power of the nerve also occurred.

In the following experiments the attempt was made to find in what way the accommodative power of the motor nerve changes in relation to the conditions of dominance between the centers, as effected by the proprioceptor impulses.

We therefore recorded normal values of the contractions of the calf muscles, by applying single square or exponential wave stimuli (capacity from 0,3 to 0,7 μF) to the sciatic plexus using the circuit of Solandt. After this an adequate stimulus to the proprioceptors of the calf muscles of the opposite side was given, by applying a load of 50 g, or by periodic stimulation of the muscles, using the Dubois-Reymond inductorium with a coil separation 11-9.5 cms, and a Bernstein interruptor.

Our experiments showed that adequate stimulation of the proprioceptors of the calf muscle, acting through changes in subordination in the centers, brings about a regular slowing of the accommodation of the motor neurons of the opposite side, and that the ratio of exponential stimulus tension to square wave stimulus tension.

These changes may be shown graphically (Fig. 1,b). We must suppose that the centers corresponding to the stretched triceps surae muscles are in a state of increased activity, in accordance with the principle of reciprocal innervation. The corresponding centers of the opposite side are inhibited, and this is shown by a slowing of the accommodation of the peripheral neuron.

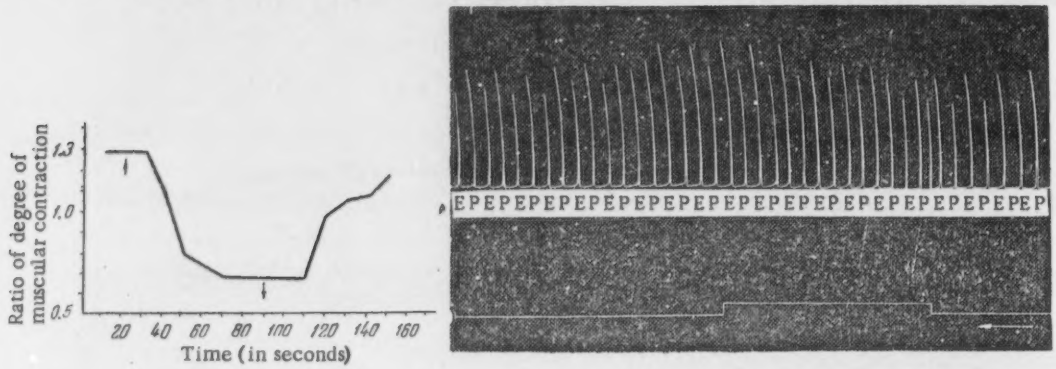

Fig. 1. Contraction of the triceps surae in the frog: a) Myogram of the triceps surae with stimulus applied to sciatic plexus. P—square wave, E — exponentially rising wave form. Traces (from above downwards): myogram, mark indicating load of 50 g applied to the triceps surae of the opposite side; myogram reads from right to left. Square wave voltage = 22, exponential wave form = 27 divisions of the voltmeter scale. Interval between stimuli 10 seconds; b) Diagram showing changes in accommodation; ↑ — application, ↓ — release of 50 g load applied to contralateral triceps surae.

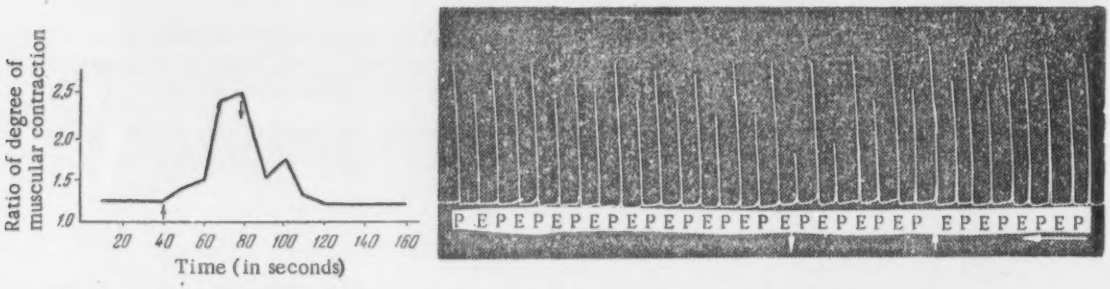

Fig. 2. Contractions in the m. semitendinosus of the frog. a) Myogram of the m. semitendinosus with stimulation of the sciatic plexus: P—square wave, E— exponential rise of voltage. Traces (from above downwards): myogram, mark showing application of 50 g load to contralateral triceps surae; ↑ —application, ↓ — release of load; myogram reads from right to left; voltage of square wave —11, of exponential wave —22 divisions of the voltmeter scale. Interval between stimuli = 10 seconds; b) Diagram showing changes in accommodation: ↑ — application, ↓ — release of 50 g load applied to triceps surae.

This conclusion is supported by the work of Kiselev, Uflyand, and Motsny who observed that during central inhibition induced by thalamic stimulation, in addition to other changes, there was a slowing of the accommodation of the motor nerve.

From P. E. Motsny's work we conclude that the slowing of accommodation in our experiment was due to a reduced lability of the muscle centers, brought about by proprioceptor influences from the muscle of the opposite side.

D. G. Kvasov and N. A. Shoshin describe similar changes in lability of the centers due to stretching the triceps surae.

In the second set of experiments we succeeded in following the accommodative changes in the motor neuron, due to changes in the subordinate relations in the centers brought about by stimulation of the contralateral antagonist.

The semitendinosus muscle was stimulated with single square wave and exponential voltages, and the calf muscle of the opposite side was stretched with a load of 50 g.

The results were always the same: the accommodation of the motor neuron increased, as can be seen from Fig. 2, a. The results are shown graphically in Fig. 2, b.

Our results agree with those described by Kvasov and N. A. Shoshin, who observed an increase in strength of the reflexes, not only of the stretched muscle, but also of its antagonist the semitendinosus, when a small tension of long duration was applied to the triceps surae.

We must suppose that the increased rate of accommodation in the motor neuron, as described in this experiment, is due to an increase in the activity, and consequent slowing of the accommodative process of the centers representing the semitendinosus.

When the m. semitendinosus of the opposite side was stretched, and the contractions of the triceps surae in response to stimulation of the sciatic plexus with the two wave forms were recorded, no clear change in the accommodation of the motor neuron was revealed.

In conclusion we may say that subordinate relationships are directly affected by proprioceptor impulses.

The accommodation of the peripheral motor nerve depends on the subordinate relationships of the centers; it is slowed by stimulation of the proprioceptors of the contralateral synergists, and accelerated by stimulation of the contralateral antagonist.

SUMMARY

Experiments with Rana esculenta have shown that the ability of peripheral motor neurons to accommodate is due to changes in the subordinate relations in the centers: accommodation is slowed if the proprioceptor of the contralateral synergist is stimulated but is accelerated when the contralateral antagonist is stimulated.

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THE EFFECT OF TEMPERATURE ON THE EXCITABILITY OF TURTLE MUSCLE

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The excitability of the conducting tissues of different animals has been the subject of many investigations carried out in D. N. Nasonov's laboratory [3, 4].

In one of these investigations a study was made of the excitability of the nerves of the turtle at different temperatures [1, 2].

In the present work we have tried to find to what extent the results found for the turtle nerve are applicable to the muscle. We used the turtle Emys orbicularis (L).

METHOD

The experiments were performed on the tonic head muscles (m. retractor capitis) and the tetanic muscles (m. puboischiotibialis) of turtles. The effect of cooling and heating between 5 and 26°C, 27 and 37°C, 30 and 41°C was investigated.

After the muscle preparation had been made, it was kept for 1½-2 hours at room temperature in Ringer's solution, and was then placed on silver electrodes in a chamber and covered with liquid paraffin, whose temperature could be measured. The thermometer was placed between the electrodes which were 15 mm apart. Stimulation was effected by discharging condensers of different capacities. In some experiments the whole of the voltage-duration curve was plotted, but usually, in order to avoid damage to the preparation from an excessive number of discharges, only the two constants a and b from Gorveg's formula $v = a/t + b$ were determined. The rheobase (constant b), i.e., the threshold for a stimulus of long duration, was measured in volts; the constant a, which is the threshold value of short acting stimuli is measured in millivolt seconds.

RESULTS

Three sets of experiments were carried out to determine the excitability of the m. retractor capitis. In the first set, the excitability on cooling from 25° to 6-7°C and reheating to 25°C was investigated.

In all cases a change of temperature produced opposite changes of excitability according to whether the stimuli were long or short. Thus, on cooling, the excitability to short stimuli was reduced by 91.2%, while that to long stimuli was increased by 16.5%; on the other hand, warming increased the excitability associated with a by 46.1% and lowered that associated with b by 24.7%. These results were statistically significant, since the mean square root deviation was less than one third of the difference between the means.

Figure 1 shows a graph of log voltage against log stimulus duration for one of the experiments. The two curves intersect at a point representing 0.1 milliseconds.

In the second set of experiments we investigated the effect of high temperatures by warming from 27°C to 37°C and cooling again to 27°C.

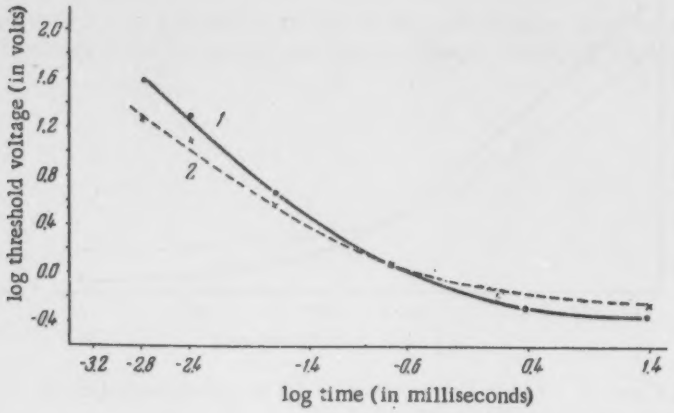

Fig. 1. Curves of log strength – log duration for the m. retractor capitis of the turtle. 1) at 6°C; 2) at 25°C.

All 11 experiments gave the same statistically significant result; heating always caused an increase of excitability to short stimuli (a was reduced on average by 23%), and a decrease of excitability to long stimuli (b increased by 52%). Cooling had the reverse effect and caused a 30% decrease and a 37% increase in excitability to short and long stimuli respectively.

In the third set, consisting of 5 experiments, the temperature was changed 30° to 41° and back from 41° to 30°.

The same results were obtained; on warming a was reduced by 20%, and b increased by 65%; on cooling 'a' increased by 31% and 'b' decreased by 38%.

Thus for any temperature change between 5°C and 41°C the two indices of excitability change in opposite directions, but the excitability of the muscle to stimuli of moderate duration, (about 0.1 milliseconds), remains unchanged and is independent of temperature.

Also when the temperature is changed from 6°C to 26°C the excitability, as indicated by the constant a changes more than does the rheobase value b , whereas in the range 27-41°C the opposite is the case.

Two sets of experiments were carried out to measure the excitability of the m. puboischiotibialis. In the first set the temperature changed from 5°C to 26°C and back to 5°C.

On heating to 26°C b increases by 41%, and a decreases by 51%. Cooling to 5° produces the reverse effect, and b decreases by 29% and a increases by 103%.

The strength duration curves from one of the experiments are shown in Fig. 2, and they can be seen to intersect at a point representing a stimulus duration of approximately 0.08 milliseconds.

In the next set of experiments, the m. puboischiotibialis was heated from 27°C to 37°C and cooled again to 27°C, just as in the case of the m. retractor capitis. The results however were not the same: this time the change of the index 'b' was very small, being between 3% and 10% which is not statistically significant, and the index 'a' decreased on warming by an average of 30% and increased on cooling by 53%. Therefore, instead of the two strength-duration curves intersecting sharply, they coincide for a considerable part of their length (Fig. 3). In other words for this temperature range the excitability of the muscle is independent of temperature for stimuli of almost all lengths.

Thus the only difference between the two muscles with respect to changes in excitability occur in the temperature range 27-37°C. Changes from 5°C to 26°C and back, affect tonic an tetanic muscles in the same way, and even the quantitative changes in the constants a and b are similar. Two strength-duration curves

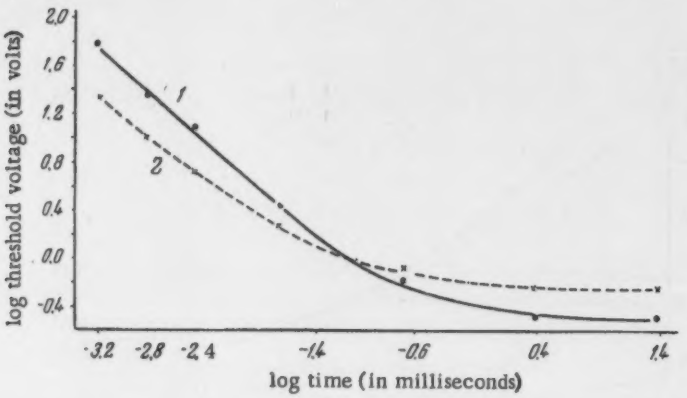

Fig. 2. Curves of log strength – log duration for m. puboischiotibialis of the turtle. 1) at 5°C; 2) at 26°C.

taken at different temperatures intersect each other, which demonstrates that the excitability to stimuli of physiological duration is independent of temperature. This reveals an adaptation of the cells to the maintenance of a constant level of excitability, unaffected by temperature changes.

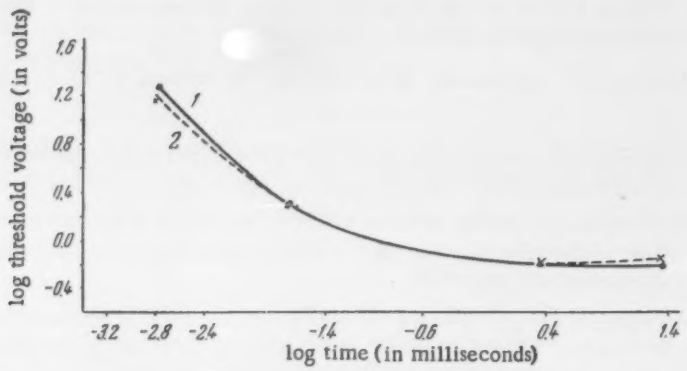

Fig. 3. Curves of log strength– log duration for m. puboischiotibialis of the turtle. 1) at 28°C; 2) at 38°C.

SUMMARY

The effect of cooling and heating of tonic and tetanic muscles of the turtle Emys orbicularis on the excitability caused by stimulation of various durations has been studied.

Cooling of muscles enhanced the excitability caused by prolonged stimulation and shortened that caused by brief stimulation. Heating caused a reverse effect. As a result, strength-duration curves cross at the area corresponding approximately to the duration of physiologic stimulus. In the zone where the curves cross the excitability is not altered by thermal changes, demonstrating an effective cellular adaptation, protecting muscular excitability from the effect of sharp thermal changes.

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THE EFFECT OF THE ESOPHAGUS ON INTESTINAL MOVEMENTS

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In contrast to the large number of reflexes which inhibit intestinal movements, and which can be obtained from certain well-defined portions of the gut, as well as by stimulation of certain afferent nerves, there are in the literature only a few references to stimulation of intestinal movements. In particular there are no references to the effect on intestinal movements of stimuli affecting the receptors of the esophagus.

Pearcey and van Liere observed inhibition of gastric contractions on distention of the esophagus, as well as a reduced tone of the cardiac and pyloric sphincters.

After dilatation of the esophagus Pearcey and van Liere found an inhibition of gastric contractions, a reduction in tone of the cardiac and pyloric sphincters, and an increase in tone of the ileocecal sphincter.

A. I. Ivanov [7] concluded from his short-term experiments on cats, that stimulation of the interoceptors of the esophagus is without effect on the motility of the intestine. I. P. Dolgachev [6] found an increased peristalsis of the large intestine after distention of the abdominal portion of the esophagus.

The aim of the present investigation was to find the effect of repeated stimulation of the esophogeal receptors on intestinal motility.

EXPERIMENTAL METHODS

The experiments were performed 18-20 hours after the last meal, on two esophagotomized dogs Malchik and Bimba, with gastric and jejunal fistulae. Stimulation of the esophagus was effected by inflating a thin-walled balloon 4-7 cm. in length, located in the middle or lower third of the esophagus. The balloon was connected to an apparatus giving a steady flow of air. It was also joined by means of a 3-way connection with a Marey capsule for registration of esophageal movements on a kymograph, and with a mercury manometer for control of pressure in the balloon. Stimulation was applied for 2-3 mins. Gastric and intestinal movements were recorded by means of balloons, using a hydraulic transmission, and a Marey capsule on the kymograph. The stimulus to the esophagus was given at various phases of the activity of the stomach and intestine. Sixty experiments were performed on the 2 dogs, during which time 448 tests of the effect of esophageal stimulation were made.

EXPERIMENTAL RESULTS

Stimulation of the mechanoreceptors of the esophagus by inflating a baloon to 40-50 ml during peristalsis of the intestine had no effect on its motility (Fig. 1); (this degree of inflation represented a pressure of 2 to 10 mm of mercury during relaxation of the esophagus, and 8-30 mm during contraction); it had an inhibiting effect on contractions of the cardiac portion of the stomach. Inflation of the baloon to 75 ml and to 100-150 ml evoked powerful contractions of the esophagus. There was little difference in the pressures obtained during the contractions, the values being 30-60 mm and 50-60 mm of mercury respectively. During relaxation, the pressures

and volumes were as follows: 75 ml - 5-20 mm; 100 ml -10-30 mm; 150 ml - 20-40 mm. There was never any inhibition of intestinal movement. The contractions of the fundus could be inhibited just as in the case of a balloon of volume 40-50 ml. The supply of air to the balloon caused no pain to the animal, for when a certain stimulus intensity was exceeded, vomitting occurred and we immediately released air from the balloon.

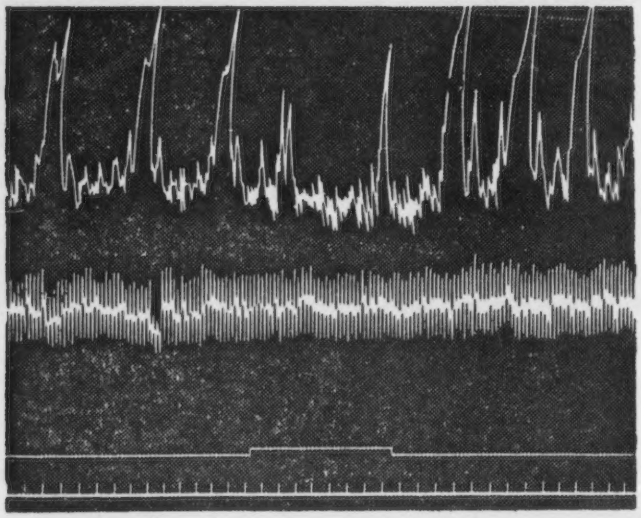

Fig. 1. Absence of inhibition of spontaneous contractions of the small intestine on stimulation of the mechanoreceptors of the esophagus, and inhibition of stomach movements. Curves (from above downwards): stomach movements, movements of the jejunum, stimulus trace (inflation of esophagus to 50 ml, pressure 8-28 mm), time (15 second intervals).

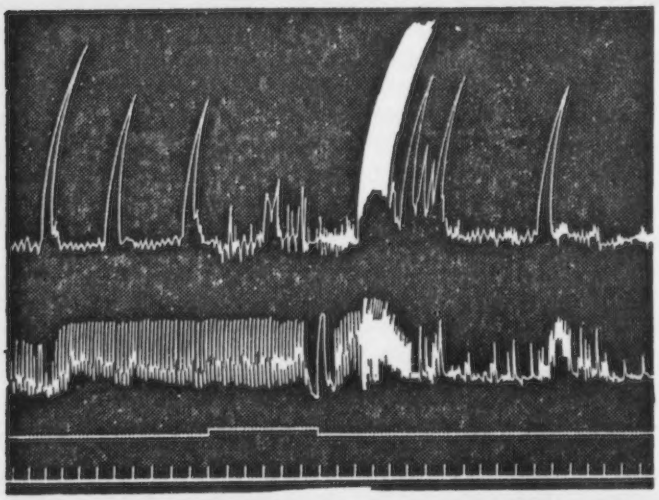

Fig. 2. No inhibition of intestinal movement at the end of the period of rhythmic activity of the jejunum, and inhibition of stomach movements. Vomitting after the stimulus. Curves as in Figure 1.

In some of our experiments stimulation of the esophagus during a period of intestinal activity resulted in tonic or peristaltic waves in the empty intestine. Stimulation of the esophagus during the terminal phase of the movement, called by O. P. Kufarev [8] the terminal rhythmic phase, (Murakami and Uchijamu's tetanic phase [10]), has no effect on the rhythmical contractions of the intestine, whatever the strength of the stimulus. A more powerful stimulus leads as before to vomitting.

In most cases a short stimulus of 1-3 minutes, with a balloon volume of 40-50 ml and a pressure of 2-10 or 8-25 mm according to the contraction of the esophagus, when the intestine was at rest, stimulated intestinal movement (Fig. 3). At the moment of inflation of the balloon, the tone of the stomach was either somewhat lowered, or unchanged. This shows that the movements induced in the small intestine do not arise from waves passing from the esophagus along the stomach.

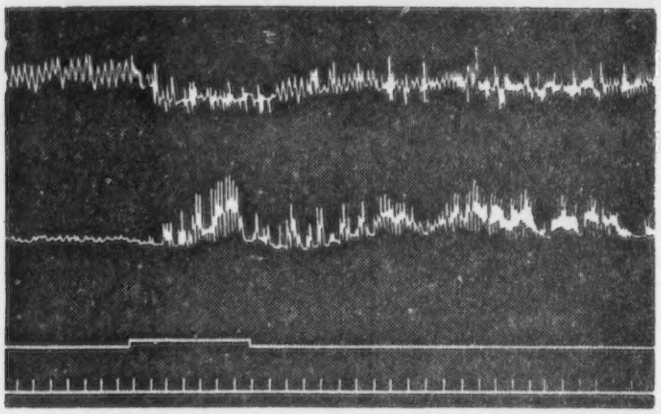

Fig. 3. Excitation of movement in the small intestine through stimulation of the mechanoreceptors of the esophagus during period of rest of intestine. Tone of stomach reduced during stimulation of esophagus. Curves as in Figure 1.

The magnitude and latent period of the response of the intestine to esophageal stimulation depends on the timing of the stimulus; at the beginning of a resting period the response is weak, with a long latent period of 1-2 minutes; if the balloon is inflated to 40-50 ml 5-10 minutes after the beginning of a resting period, the response is still weaker or absent, but if the stimulus is applied later, the response is greater and the latent period is shortened to 30-60 seconds. As the moment at which the esophagus receives the stimulus approaches the period at which movement would begin spontaneously, the response increases in amplitude and duration, and the latent period is reduced. Stimulation with 40-50 ml just before an active period is due, brings on contractions which last for a considerable time and which represent a normal period of activity.

Thus the reaction of the small intestine to stimulation of the esophagus, is the same as that which we previously observed to originate from the stomach [4]. On stimulating the esophagus with 75-100 ml during a period of intestinal rest, the same reaction as before occurs, except that the latent period is shorter, and the response is greater. If the stimulus is very strong it causes vomitting, just as in the case when it is applied during a period of activity.

If the stimulation is applied many times per day, an adaptation occurs to the particular strength of stimulus used, and the response is reduced. Increase of stimulus strength again evokes a clear-cut reaction.

The results afford clear evidence of a stimulant effect exerted by the esophagus on the movement of the small intestine when an adequate stimulus is applied, for a dog is capable of swallowing a lump of food of greater volume than the inflated balloon which we used. We cannot therefore agree with A. I. Ivanov who concluded from the evidence obtained in short experiments, that no changes in intestinal movements occur during the passage of food along the esophagus.

It is well-known that intestinal movements are considerably reduced for a considerable period after opening the abdominal cavity. This is particularly true of the intestine of the cat, in which the chief movement is a rhythmical one which divides the gut up into segments [9]. In the operations performed by A. I. Ivanov, considerable damage was caused to the stomach and intestine, which must certainly have affected intestinal motility. We must also remember that these experiments were performed on anesthetized animals, and this may be the reason why he [7] failed to observe the stimulant action of the esophagus on intestinal motility. In his experiments stimulation of the mechanoreceptors of the esophagus produced no inhibitory effect on the intestine, and this agrees with our results. Since stimulation of the esophagus exerts its action on the intestine at a time when the gastric tone has been reduced also by esophogeal action, or else has remained unchanged, we deduce that there must exist some reflex not yet described in the literature, which mediates this effect. Additional evidence is obtained from our results showing a stimulant action of the esophagus on intestinal motility in cases where the intestinal wall was cut above the place from which the movements were recorded.

From these results and those of the previous investigations [1-5], we may conclude that the increase in intestinal movements that takes place on feeding in normal animals, occurs as a result of the summation of the following reactions: 1) conditioned reflex stimulation from the eye and nose reacting to the sight and smell of the food, before feeding. 2) conditioned reflex stimulation initiated by the act of eating [5], 3) reflex action from the esophagus, 4) reflex action from the stomach. Also, on entry of chyme into the intestine, the degree of movement of the small intestine is to a large extent determined by the action of its constituents on the duodenum and other parts of the intestine.

SUMMARY

Extensive observations of two esophagotomized dogs with fistulae of the stomach and jejunum have shown, that stimulation of the esophagus by an introduced ballon elicits reflex movements of the jejunum. The character of reflex activity depends on the intensity of stimulation and the functional state of the motor apparatus of the stomach and intestine at the moment of esophageal stimulation.

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EFFECT OF CAFFEINE ON SUMMATION IN THE SUBMAXILLARY GLAND

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The action of caffeine on the secretion of the salivary glands is of interest for two reasons; first, because of its effect on the summation of stimuli, and secondly because of its action on the gland itself, this being the last stage in the conditioned food reflex, from which inferences are made about the basic processes in the cerebral cortex.

The present work is a study of the effect of caffeine on summation in the submaxillary gland.

METHOD

Thirty-one experiments were performed on cats. A combined anesthetic was used; induction was with ether which was followed by injection of a 0.25-0.3 g per kg body weight of a 2% solution of Medinal.

The gland was separated from the nervous system only, by cutting the lingual nerve, and the preganglionic fibers of the sympathetic nerve supply. The rate of secretion was recorded from the movement of the liquid meniscus in a glass tube connected to a fistula in the duct of the gland. In each case, the ability of the gland to summate was determined from the number of electrical stimuli to the gland required to cause secretion before and after the administration of caffeine. Intravenous injections were given of coffeinum benzoicum in a 2-10% solution; in 10 experiments the amount was 0.0067-0.062 g and in 6 experiments it was 0.1 g per kg body weight.

RESULTS

The action of caffeine could be observed as early as five minutes after its injection. In all the 16 experiments, the number of stimuli required to elicit the secretion was less after the injection (Table 1).

For example in Experiment 10, with stimulation at one second intervals, before the injection of caffeine, 98 stimuli were required but after the injection only 20 were needed; at 4 per second, before injection 42 and after injection 22 stimuli were required.

With lower rates of stimulation, caffeine also caused a considerable reduction in the number of stimuli required. In the range from 8 stimuli per second to 1 stimulus per 2 seconds, the slower the rhythm the greater was the reduction.

In 8 experiments there was a displacement of the minimum rate towards a lower value. Thus, in Experiment No. 1, before caffeine the minimum rate was 4 per second, and after, 1 per second.

In the second set of experiments we measured the greatest interval between electrical stimuli at which summation occurred (Table 2).

TABLE 1

The Effect of Caffeine on the Secretory Reaction of the Gland in Response to Electrical Stimulation

Expt.No.	Strength of Stimulus (distance between the induction coils in cm.)	Rate of stimulation (No. of stimuli required to cause secretion	Minumum number of stimuli required to cause secretion	
			Before injection of caffein	After injection of caffein
1	17	1/1	42	25
		2/1	44	24
		4/1	38	30
2	25	2/1	120	48
		4/1	138	94
		8/1	112	88
6	24	1/2	50	27
		1/1	35	24
		2/1	29	19
		4/1	30	22
		8/1.	40	24
10	25	1/1	98	20
		4/1	42	22
		8/1	44	28
11	15	1/1	28	5
		2/1	24	8
		4/1	32	11
		8/1	46	22

After the injection of caffeine the gland was able to summate the effects of stimuli, at intervals which before the injection were too great to be effective. After caffeine the maximum interval between the stimuli was increased 3-5 times.

We have found no references in the literature to the effect of caffeine on summation or other functions of the salivary glands.

As our work has shown, caffeine increases the reactivity of the gland, as is shown by its increased power to effect summation. We found no clear relation between the summation effect and the dose; the dose range was very great, from 0.0067 to 0.1 g per kg body weight.

TABLE 2.

Effect of Caffeine on the Maximum Interval between Stimuli at which Summation is Effective

Expt. No.	Interval between stimuli (in seconds)	
	Before injection of caffein	After injection of caffein
19	1	3
21	1	3
22	1	8
25	1	4
26	2	12
28	2	10
29	2	20
11	2	15
17	3	10
20	3	12
18	4	5
13	4	5
24	5	20
31	6	24
31	7	20

SUMMARY

In cats with the lingual nerve and the preganglionic sympathetic nerve trunk dissected intravenous injection of caffeine enhanced the ability of the submaxillary gland to summate induction current stimuli eliciting a secretory effect.

THE REFLEX MECHANISM OF THE SPECIFIC DYNAMIC ACTION OF FOOD

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The question of the specific dynamic action (SDA) of food has been of interest to many for a long time. Most workers [10, 11, 12] consider the SDA to result from the direct action of the food breakdown products on the body cells. However, since 1920, facts have begun to accumulate which suggest that the nervous system is involved. It has been shown [8, 9, 13], that after section of the gastric nerves and also after giving ergotamine, the SDA is reduced or disappears completely, while after treatment with substances exerting a stimulant action on the autonomic system (mainly the sympathetic), it is increased.

The role of the cerebral cortex in SDA has been shown by K. M. Bykov's collaborators [3, 4, 6]. R. P. Olnyanskaya [4] distinguished two phases of the SDA: a complex reflex, and a neurochemical. However she and other of K. M. Bykov's co-workers treated the reflex phase as a pure conditioned reflex. The reflex component of the neurochemical phase has scarcely been studied at all. In E. A. Asratyan's laboratory much data has been obtained from decortication experiments on the effect of the cerebral cortex on unconditioned reflex regulation of gastric, biliary and salivary secretion, regulation of the circulatory system, etc.

We studied particularly the SDA of food in dogs, after extirpating the cortex of one or both cerebral hemispheres.

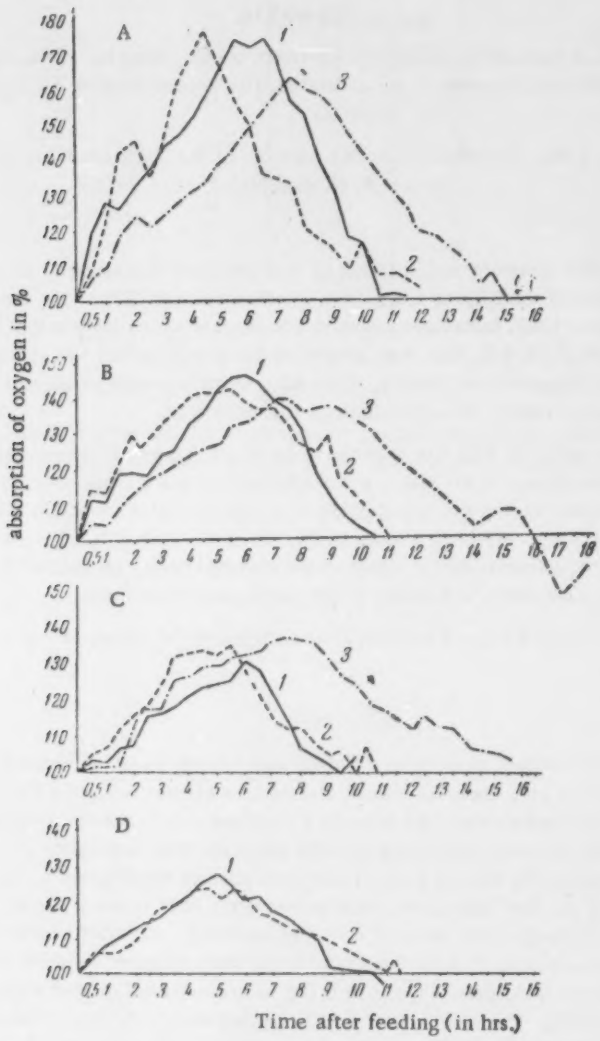
METHOD

Gaseous exchange was determined by Douglas-Haldane and Tsuntts-Haldane methods. The animals were given 200 g of meat 18-20 hours after their last meal. The expired air was collected for 30 minutes before giving the food, and for 5-10 minutes every half hour for 12-18 hours. At each stage of the investigation, (i.e., before the operation, and after removal of one and then the other cerebral hemisphere), each animal was tested in 8-15 experiments to determine the SDA of food. In order to prevent conditioned reflexes being formed by the experimental set-up in which the food was given, these experiments were randomly mixed in a series, and determinations of the gaseous exchange were made in a resting condition, and after administration of thyroxin. Also at each stage of the investigation, 2-3 control experiments were arranged in which the gaseous exchange was determined on fasting animals (in these experiments the variation in the gaseous exchange during the course of the day was not more than 5%). These experiments were carried out on the dog Prokaznik for 6 months before the operation, for 8 months after the extirpation of the first cerebral hemisphere, and for as long again after the removal of the second; on Beloi the corresponding periods were $4\frac{1}{2}$, 3 months and one year; on Novy the periods were 5, $8\frac{1}{2}$ months, and 1 year; on Umnits – 8 and $8\frac{1}{2}$ months (the dog died 5 days after removal of the second cerebral hemisphere). Also on one dog Tsygan, the experiments were performed only after bilateral decortication (6 experiments after 1 month, and 6 after 1 year 11 months). Since we had no SDA curves for this animal before the operation, it was difficult to determine how much the values obtained later differed from

the normal, and these latter could only be used to find the changes in SDA which occurred after the bilateral decortication.

RESULTS

We give here the average values of the gaseous exchange calculated from all the experiments for each dog. In normal animals, even half an hour after taking food the oxygen consumption is increased: in Prokaznik the average increase was 19.6%, in Beloi –11.1%, in Umnits –4.4%, in Novy – 3.4% (see Figure). The maximum oxygen absorption occurred in Beloi and Novy 6 hours, and in Umnits 5 hours after feeding. In Prokaznik there were two almost equal maxima after $5\frac{1}{2}$ and $6\frac{1}{2}$ hours. The increase in oxygen consumption was 74.4% in Prokaznik, 46.1% in 30.3% in Novy, and 27.1% in Umnits. In Prokaznik and Beloi there was a return to



Changes in the oxygen absorption rate after feeding and after decortication.
A – Prokaznik, B – Beloi, C – Novy, D – Umnits. 1) Before operation, 2) after removal of one cerebral hemisphere, 3) after removal of both cerebral hemispheres.

the initial exchange rate after $10^1/_2$ hours, and in Novy and Umnits after $9^1/_2$ hours. There were thus considerable individual variations in the reactions to food of the different dogs, and a comparison of the SDA before and after decortication was only possible by comparing pairs of values for the same animal.

After removal of one cerebral hemisphere in almost all the experiments there was an increase in the period of raised oxygen consumption. The maximum absorption of oxygen occurred somewhat earlier, and had approximately the same value as that obtained before the operation.

In completely decorticate animals considerable changes in the oxygen consumption curve were shown. The increase half an hour and one hour after feeding was less than before decortication. This was particularly clearly shown Proksaznik and Beloi. Before the operation in Prokaznik the exchange rate had increased by 19.6% half an hour after feeding, and by 27.5% one hour after feeding, while after the operation the figures were 6% and 9% respectively; Beloi the figures were 11.1% and 10.3% before, and 4.5% and 4.4% after operation. In Novy the changes were not so distinct because even before the operation the increases half an hour and one hour after feeding were only 3.4% and 2.7%. The gaseous exchange curve in Prokaznik and Beloi lies below the preoperational curve for several hours. The maximum rate was delayed by operation: in Beloi and Novy by $1^1/_2$ hours, and in Prokaznik by not less than 1 hour. The following was the most noticeable effect: in the normal animal the gaseous exchange rate returned to the resting level after $9^1/_2$-$10^1/_2$ hours, but after complete decortication only after 15-16 hours. No regular change in the maximum rate was observed (see Figure).

The experiments on the SDA of food were begun a few days after the complete decortication, and were continued for the whole of the period of investigation. However there was no appreciable change in the shape of the oxygen consumption curves during this time.

What body functions affecting the SDA are absent after decortication? K. M. Bykov's co-workers showed the predominantly conditioned reflex nature of the first phase of the SDA. But the changes observed by us could hardly be explained by the disappearance of conditioned reflex regulation. One has only to recall the shape of the SDA curves obtained after introducing food directly into the stomach [3, 4]. In A. R. Makharova and R. P. Olnyanskaya's experiments and in our own, the gaseous exchange curve began both to rise later and to reach a maximum later than in normals; on introducing food into the stomach, the delay was considerable (3-5 hours), whereas in decorticate dogs the gaseous exchange rate began to rise even in the first hour and reached a maximum $1^1/_2$-$2^1/_2$ hours later than normal. It is clear that this represents the unconditioned reflex mechanism of the first phase of the SDA, changed admittedly by the effect of decortication. The part played by the conditioned reflex component in the second SDA phase is minimal. Nevertheless the considerable changes in the second phase which occur in decorticate dogs certainly are a result of the loss of the cortex, and resemble closely changes in other reflexes found recently by other of E. A. Asratyan's co-workers [1, 2, 7] in decorticate animals.

We may suppose therefore that the reflex mechanism is involved in the second phase of the SDA as well, while observing at the same time that the unconditioned reflex regulation becomes less perfect and precise after decortication. The reflex response to the food stimulus (increased gaseous exchange rate) is unsteady for some time, and is then maintained for a considerable period. It seems to us that these results are in line with the Pavlovian view of the cortical representation of unconditioned reflexes.

R. P. Olnyanskaya showed the importance of thyroxin in the SDA. The question of the degree to which the cerebral cortex is involved in the mechanism of the action of thyroxin is itself of considerable interest. This stimulated us to investigate in our own dogs the effect of thyroxin on the gaseous exchange rate. Subcutaneous injections of 0.3 mg per kg body weight of thyroxin were given. The expired air was collected before giving the hormone, and thereafter for half an hour three times for 5-9 days.

Before the operation, in all dogs, for the first day after the thyroxin injection, the gaseous exchange rate was increased by 3.6-8%. The maximum increase occurred on the second, or more rarely on the third day, and varied from 23.9 to 55.3%; by the 4th-5th day the rate had returned to its original value. After removal of one hemisphere the change in the exchange rate was not clearly shown, and was not always in the same direction.

In all dogs, after complete decortication, the rate of absorption of oxygen increased more after injection of thyroxin than normally.

The greatest increase in comparison to the normal was shown in the increase in energy expenditure per kg of body weight; this is shown in the following table:

Dog	Percentage increase before operation	Percentage increase after operation
Prokaznik	55.2	90
Novy	36.6	69.8
Beloi	51	60.5

In all the animals a phenomenon was shown, which occurred in all the other functional tests. After giving thyroxin to decorticate animals the gaseous exchange rate returned to its original level 2-3 days later than in the same animals before removal of the cortex.

The results obtained show that the cerebral cortex plays an essential part in the effect of thyroxin on the gaseous exchange rate.

SUMMARY

Specific dynamic action of food was studied in normal dogs and after extirpation of the cortex of one or both hemispheres of the brain. After complete decortication the curve of the specific dynamic action of food slowly rises, then reaches the top and returns to the initial level after a much longer time than before the operation. These changes indicate that the cortex participates not only in conditioned but also in unconditioned reflex mechanism of specific dynamic action of food.

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EFFECT OF REPEATED MUSCULAR WORK ON PULSE RATE AND BLOOD PRESSURE

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The results of many years of experience and a mass of experimental results lead us to conclude that frequently repeated muscular work is necessary in order to establish a condition of being in training, and that after the muscle work, a trace (aftereffect) remains, on which the functional changes produced by subsequent exercise are superimposed.

In a more detailed study of the aftereffect some authors have investigated the functional changes associated with repeated muscular work, in the case when the interval between the repetitions is small.

The group directed by G. E. Vladimirov [1, 2, 3] showed in animal experiments, that on repetition of a standard exercise at short intervals the increase in the lactic acid content of the blood was less at each repetition. With repeated exercise in men they failed to find a regular reduction in the "working shifts" of lactic acid. There were however certain changes associated with the repeated work: in many cases there was a reduction in the respiratory quotient (R.Q.) and a more rapid return of the oxygen consumption to normal after each successive period of work.

The work of M. E. Marshak [4, 5] showed that a second spell of work, undertaken before complete recovery from the first, does not result in either a further expenditure of energy of an increase in the blood lactic acid, and that in the next spell of work the period of "working in" is shortened.

The effect of repeated muscular work on the pulse rate has been described in the papers of I. N. Popov and G. G. Saruchanov [6].

Our aim was to find, through study of respiration and heart rates in repeated muscular work: a) the relation between the initial work, and changes in subsequent performance, carried out at short time intervals in the same experiment, b) the effect of frequent repetition of a standard set of exercises.

METHOD

The investigations were carried out on subjects aged 18-25 years. Records were made of the pulmonary ventilation (every thiry seconds), the respiration rate, the pulse frequency (using a photoelectric counter), the blood pressure, and the degree of oxygen saturation of the arterial blood (oxyhemometer).

The dynamic work was done on a bicycle ergometer, and the static work was performed on an apparatus in which a constant tention had to be maintained.

After a certain level of training had been reached in one task, another was introduced, and after 10-15 training sessions, a third was added. In certain cases four separate exercises were used in one experiment.

The average interval between dynamic work sessions was 5 minutes, so that they occurred at the end of a period of recovery from the work of the preceding session. In one series the interval was increased to 20 minutes. Repetition of the static work was carried out after complete recovery, as shown by the return of all the measured quantities to their initial values (2-8 minutes according to the subject).

RESULTS

On repetition of two or three spells of dynamic work, each successive working session caused a smaller increase in pulmonary ventilation, pulse frequency and respiration rate; in addition there was a more rapid increase of these quantities at the beginning of the session and the recovery period was shorter than in the preceding session (Fig. 1).

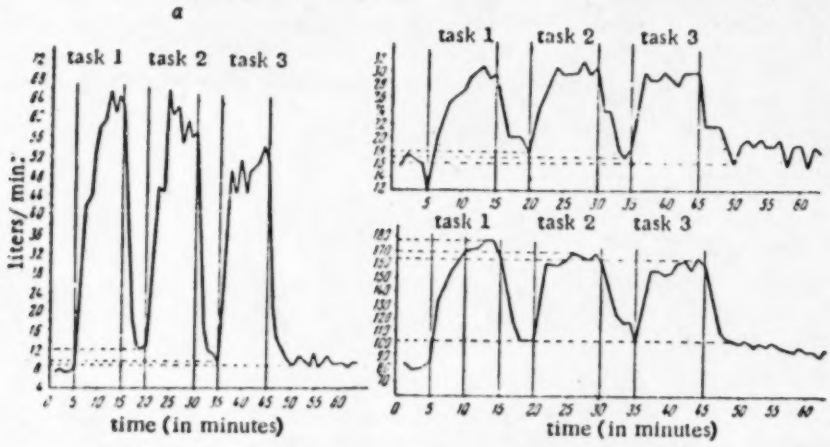

Fig. 1. Effect of one task on the pulmonary ventilation (a), respiration rate (b), and pulse frequency (c), during the performance of the next task.

During the first working period, the arterial oxygen saturation was reduced in some subjects by 3-6%, and in each subsequent session the reduction became progressively smaller.

Although the pulmonary ventilation was less in the first than in the second and third sessions, the oxygen saturation was increased. This may indicate that the oxygen saturation does not depend on the pulmonary ventilation rate, but on the relative values of the pulmonary circulation and gaseous exchange rates.

The respiratory and circulatory changes occurring during the 2nd and 3rd working sessions of one experiment, were analogous to the changes observed after many days of training to a single task.

During the process of training to a repeated set of tasks, it was noticed that the pulmonary ventilation, the respiration rate, and the pulse rate during the first task, all increased gradually in each successive experiment (Fig. 2, a and b). The shape of the recovery phase after the first session also showed a change in all of the quantities measured: during the first and second minutes after the end of the first working session there was a considerable reduction in pulmonary ventilation, respiration rate, and pulse frequency; however, subsequent recovery was greatly slowed or even arrested. In some experiments, even 1-2 minutes before the start of the next task, an increase in pulmonary ventilation and pulse frequency was observed.

This phenomenon is observed only on repeatedly working through a group of exercises, and is explained in terms of the emergence of a dynamic stereotype, in which all the tasks which make up the group, and the intervals between them become fixed as a unit in the higher nervous centers, and from the onset the work is performed as one single complex.

This conclusion receives support from the second set of experiments, in which the severity of each separate task in the group was periodically changed according to a single accurately followed plan. In the early experiments the task of altered severity caused fluctuation of the pulmonary ventilation, respiration rate, pulse rate,

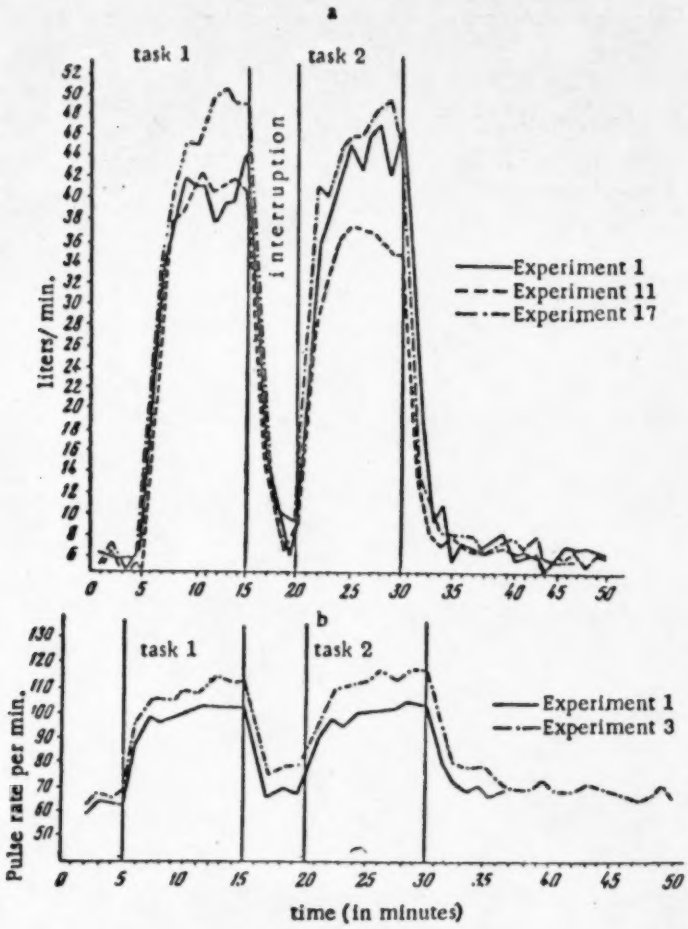

Fig. 2. Effect of training on a group of repeated standard tasks on pulmonary ventilation (a) and pulse frequency (b).

and arterial oxygen saturation of varying extent, according to the degree of alteration. The fluctuation of these quantities increased in proportion to the extent of the training.

The following experiments offer good evidence that the changes in the observed parameters occurring on repeated execution of a group of tasks, are due to the development of a dynamic stereotype: if, after long training to a group of tasks whose severity changed during the experiment, the severity was made not to change, there then occurred just such fluctuations in pulmonary ventilation as are normally observed to occur with work tasks of varied severity. Typically the fluctuations of pulmonary ventilation coincided in time and intensity with the periods of greater or less work (Fig. 3).

Repetition for several days of certain static exercises with short rests, also led to the development of a dynamic stereotype.

The breakup of the habitual complex of static tasks by an extra task, or by the change of one of the intervals, caused respiratory and circulatory changes to occur while working on these standard tasks; the pulmonary ventilation, the respiration rate and the pulse frequency increased in all tasks, and in spite of the increased pulmonary ventilation, the standard static load caused an increased fall in the arterial oxygen saturation. This indicates a breakdown of the correlation between aeration and the pulmonary blood supply of separate parts of the lungs, and a reduced effectiveness of the respiration.

The results of these investigations afford convincing evidence of the highly important function of the higher nervous centers in effecting the respiratory and circulatory changes induced by both repeated muscular work during the course of a single experiment, and by repeatedly working through a set of muscular exercises.

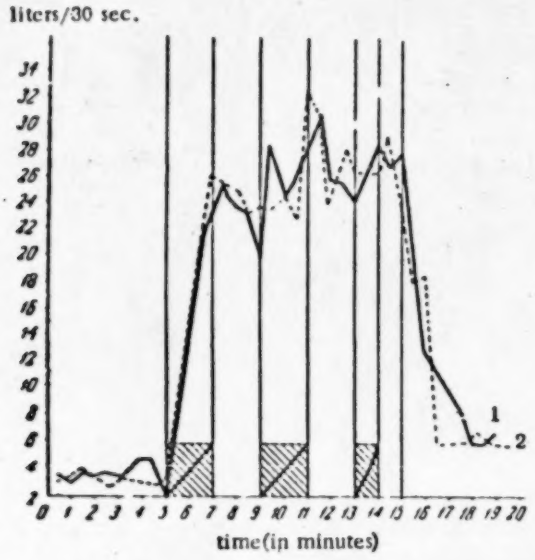

Fig. 3. Changes in pulmonary ventilation while performing work at a constant rate after lengthy training on work at a variable rate.
1) Task No. 1; Task No. 2.

SUMMARY

Changes in lung ventilation, frequency of respiratory movements, pulse rate, blood pressure and oxygen saturation of arterial blood have been studied during dynamic and static work.

With each new performance lung ventilation, pulse and respiratory rate increased less and less.

The rate of these functions was increased with the start of each new performance, while the duration of the restoration period diminished.

As training in each complex of work was prolonged, lung ventilation, pulse and respiratory rate, increased with each experiment: the character of their restoration changed as well.

Changes of external respiration and blood circulation occurring during work resulted in the development of a reinforced dynamic stereotype.

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CHANGES OF THE OCULO-VAGAL REFLEX IN MONKEYS DURING PHARMACOLOGICALLY INDUCED SLEEP

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D. I. Miminoshvili [3] observed in monkeys suffering from a neurosis, or hypnotized into a slumbering or an alert condition, changes in the electrocardiograph indicating disturbance of the coronary circulation. Analogous changes have been described in hypnosis in man [6, 7]. Clinical observations have indicated the occurrence of cardiac crises [4,8] and the occurrence of unpleasant sensations in the region of the heart [2] during short spells of sleep. In this connection it would seem worth-while to investigate the oculo-vagal reflex in monkeys during pharmacologically induced sleep, and to attempt to explain changes in the reflex which occur during the various phases of cortical inhibition found in studying the relation between the cortex and the lower centers in various cortical conditions.

METHOD

After a preliminary study of the oculo-vagal reflex in monkeys, we investigated the same reflex in barbamyl*-induced sleep in 6 rhesus monkeys, including 3 practically healthy animals (Muruk, Dergach, Muflon) and three suffering from various pathological conditions (Karabas—clearly shown neurosis, Tiger— prolonged hypertension, and Fata— marked vagotonia, and fatness due to long confinement in a small cage).

A subcutaneous injection of 80-85 mg per kg body weight of 10% barbamyl was given in the spinal region. Sleep lasted about 4 hours. The experiments were carried out in a sound-proof room, and an electrical recording of the pulse rate was made. Lead 2 of the electrocardiogram was by needle electrodes and the recording made on an ink recorder. After the animals had become used to the experimental setup , and the baseline of the normal rhythms had been established and recorded, a record was made of the oculo-vagal reflex when the animal was alert, drowsy, in deep sleep, or waking.. The reflex was elicited every 10-15 minutes by pressure of the finger on the right eye for 45 seconds. For each of the phasic conditions, pressure was applied for an average of 4 times. The strength of the reflex was measured as the percentage change of the initial heart rate caused by each application of pressure to the eye. The value for the initial strength of the reflex in the alert condition was taken as the mean of several determinations.

RESULTS

Shortly after the barbamyl injection, we observed in the healthy monkeys, over a period of 20-30 minutes, a gradual increase in the value of the oculo-vagal reflex from its value in the alert state. As the sleep became deeper the value of the reflex progressively fell to zero. This second phase of the induction of sleep lasted about 20 minutes.

* Russian trade name.

During deep sleep the reflex increased somewhat in strength and attained a value intermediate between that of induction and awakening periods. During the 2nd half of the period of sleep the reflex attained or slightly exceeded the initial value. On arousal the strength of the reflex was as a rule at first greater and then fell to its original value.

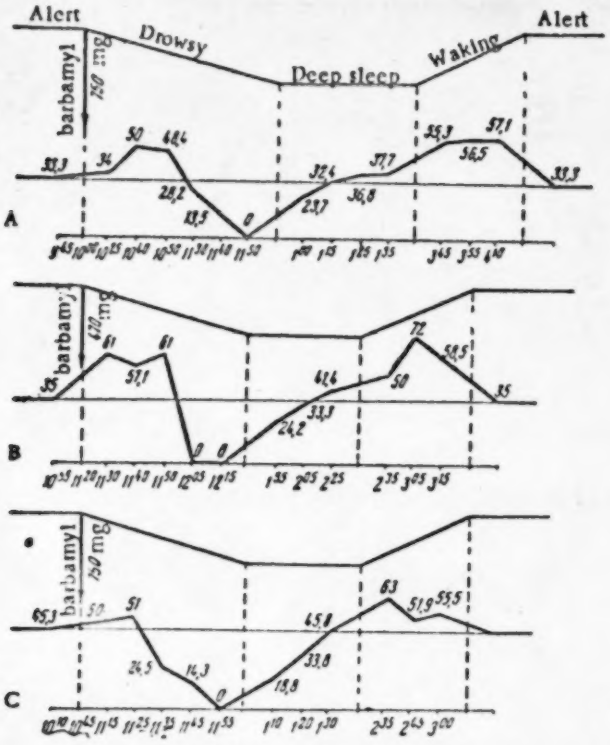

Fig. 1. Changes in the oculo-vagal reflex in normal monkeys. A) Muruk, Experiment 6/15/1955; B) Muflon, Experiment 9/23/1955; C) Dergach, Experiment 6/14/1955; Strength of the reflex expressed as a percentage of its original value for each application of pressure to the eye. Below – time in hours and minutes.

This sequence of changes in the oculo-vagal reflex during the different phases of cortical inhibition was repeated as a rule in all the normal animals (Fig. 1). The increase in the strength of the reflex during the first half of the period of induction and on arousal was found to be the same in each of the two groups. Nevertheless the pathological group showed several differences (Fig. 2). Although the reflex gradually diminished during the second half of the induction period, it did not become reduced to zero. In the second half of the sleep period the reflex value was stronger than at first, though more frequently it had a strength somewhat below the maximum value characteristic of the period of arousal.

The series of changes just described in the strength of the oculo-vagal reflex during the different phases of sleep can be explained in terms of the relations between the cortex and the subcortex.

In the first half of the period of sleep induction, we may suppose that the increase in the strength of the reflex is brought about by positive induction from the cortex, where the inhibitory process begins to become concentrated on the subcortical centers and on the vagal nuclei in particular. The progressive weakening or inhibition of the reflex in the second half of the induction period corresponds to the process of gradual irradiation of the inhibitory process from the cortex into the subcortex. The growth of the reflex during the period of

deep sleep is a result of the development and increase in strength of the process of disinhibition; this commences in the lower regions of the subcortex, including the vagal nuclei, and later reaches the higher subcortical centers as a result of a weakening of the inhibition associated with sleep. At the end of sleep the inhibitory process in the cortex has become weakened to such an extent that it is no longer able to counteract the growing strength of the disinhibitory process in the subcortex, and it is evidently this which leads to awakening. We may suppose that the increase in strength of the reflex is brought about by positive induction from the cortex into the subcortex which has been released from the effects of inhibition.

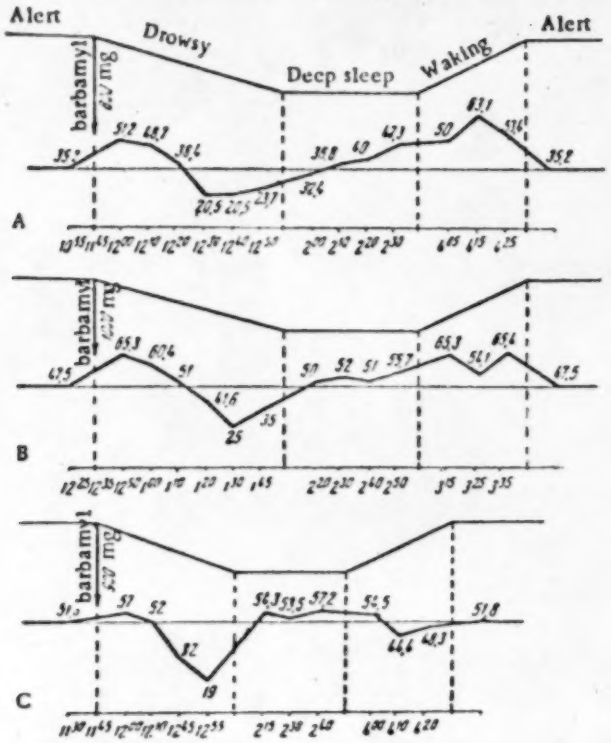

Fig. 2. Changes in the oculo-vagal reflex in monkeys with pathological disorders. A) Karabas, Experiment 9/15/1955; B) Fata, Experiment 11/20/1954; C) Tiger, Experiment 10/6/1954. The strength of the reflex is expressed as a percentage of its initial value for each application of pressure to the eye. Below – time in hours and minutes.

These explanations agree entirely with I. P. Pavlov's view [5], that mutual induction occurs during changes in the condition of sleep or wakefulness.

The differences between the changes of the oculo-vagal reflex in the two groups is probably due to a disturbance of the processes of mutual induction, brought about by a weakening of the inhibitory power of the cortical cells, and disruption of the normal central regulation of autonomic function, as a result of the neurotic imbalance in the affected group. The different forms of insomnia which are present in nervous disorders can probably be explained in the same way. It is therefore necessary in such patients to strengthen and deepen the necessary concentration of the inhibitory process in the cerebral cortex, by sleep therapy.

Our experiments showed that the strength of the unconditioned oculo-vagal reflex depends on the extent to which the inhibitory processes are distributed over the different levels of the brain during sleep, and on the inductive relationships between the cortex and the subcortical centers. At the same time the oculo-vagal reflex may

serve as an index of the depth of sleep, and the changes it undergoes may reveal the functional relationship of cortex to subcortex both in health and in various functional nervous disorders.

SUMMARY

The investigation was conducted on six rhesus monkeys—three normal and three neurotic. Sleep was induced by hypodermic injections of barbamyl. The oculo-vagal reflex (Aschner reflex) was electrocardiographically registered when the monkeys were awake, falling to sleep, during sleep and when waking up. The magnitude of each reflex elicited by pressure on the right eye was rated on a percentage scale of the initial reflex rhythms.

Typical alterations of the reflex change of the heart rhythm were found for the above-mentioned stages of sleep and wakefulness.

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CHANGES IN RENAL EXCRETORY FUNCTION IN ANIMALS WITH SEPARATE EXTERIORIZED URETERS

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(Received April 21, 1956. Presented by Active Member of the USSR Academy of Medical Sciences V. N. Chernigovsky)

A considerable degree of renal functional lability and asymmetry has been observed by several authors.

In K. M. Bykov's laboratory [2, 3] the unequal excretions of the two kidneys were observed in spontaneous diuresis, particularly after an increased water intake.

M. I. Olshansky [6] found a difference in the performance of the two kidneys on injecting various pharmocologically active substances. Functional asymmetry has also been reported by A. A. Pavlovskaya [8], A. L. Komendantova [4], V. L. Balakshina [1], and N. A. Myacoedova [5].

However there is some evidence in the opposite direction. S. Streyter [14] and E. Allard [11] conclude from urological investigations, that both human kidneys function identically and excrete the same quantity of urine. The same view, based on animal experiments, is held by A. R. Cushny [12], and M. Ercole [13]. G. M. Shpuga, having followed changes in diuresis in three dogs, admits the possibility of small differences between the performances of the two kidneys, but considers these to be due only to chance variations.

In studying variations in renal excretory function in animals with separately exteriorized ureters, we usually found unequal amounts of urine excreted by the right and left kidneys. The aim of the present investigation was to find what conditions were relevant to this phenomenon.

METHOD

The experiments were carried out on two dogs with the ureters separately exteriorized by Orbeli's method [7]. Altogether 50 experiments were performed. The conditions were maintained as nearly as possible unchanged throughout the course of the experiments. The feeding routine was always approximately the same. The experiments were performed in the morning and always when the stomach was empty (16-18 hours after feeding). In all experiments, measurements were made of spontaneous diuresis, and diuresis after drinking 40-60 ml per kg body weight of diluted milk. Any one animal always received the same amount of fluid.

In the group of experiments on the action of mercuzal* the animals were given a load of water and milk.

RESULTS

In both dogs the two kidneys each maintained a constant rate of excretion, though there was a difference in the rates between the right and left sides. This functional asymmetry was fairly stable. Usually during a series of experiments, one kidney would predominate. However in some cases the kidneys as it were exchanged rates, not only on different days but within the period of a single experiment.

* Russian trade name.

In the case of spontaneous diuresis, the difference between the volumes of the urine was comparatively small. In the dog Zlacliki, the right to left volume ratio, averaged over a three hour period, varied from 0.87 to 0.95, and in Liski from 1.08 to 1.15.

In the experiments with the water-milk load, or with this load given together with mercuzal, the difference between the two amounts of excretion was increased. After the load had been given, the increase in excretion was different on the two sides, the ratio being 1.6, while after intramuscular injection of 1 ml of a 10% solution of mercuzal together with the fluid load, the ratio rose to 2.0.

If we compare the amounts excreted by each kidney over the relatively short time interval of 15-30 minutes instead of 3 hours, the ratio rises to 2.68.

The chemical composition of the urine excreted by the right and left kidneys was also different. This has been confirmed by the reports of R. O. Faitelberg and A. A. Shapiro [9] and N. A. Myacoedova [5].

According to our results, there was an appreciable but unimportant difference in the chloride concentration of the urine from the two sides. There were also differences in the creatinine concentrations. Thus, for example in pairs of urine samples from the two sides, the creatinine contents were 22 and 24 mg%; 17 and 10 mg%; 28 and 21 mg%, and so forth. Calculation using Reberg's formula suggests that there is a change in both glomerular filtration and tubular reabsorption rates as well as a change in their ratio. The asymmetry did not however prevent the kidneys from being affected as a pair. Their excretion rates were increased or decreased together.

In an attempt to explain the reason for the asymmetry in normal kidney function, we investigated the effect on excretion of partial or complete suppression of the regulatory centers, by means of narcotics, on the assumption that difference in rates between the two sides was due to influences originating in the central nervous system.

For this purpose morphine and chloral hydrate were used. The morphine dose was 1-2 ml of 1% solution injected subcutaneously, and chloral hydrate was given in doses of 0.75-3.0 g together with the fluid.

The subcutaneous injection of morphine caused a reduction in diuresis in all cases. For the first 15-30 minutes after the injection the asymmetry remained unchanged, or even increased somewhat. This coincided with the period of morphine stimulation. Subsequently the difference between the rates on the two sides was considerably reduced, and the two quantities of urine excreted became almost identical. These results lead us to suppose that the functional asymmetry results from the functional condition of the central nervous system.

The results obtained with chloral hydrate also deserve consideration. Shortly after the animal had been given the chloral hydrate, it fell asleep. During sleep, the diuresis of the water-milk load was increased, though the increase was less than in the control experiments in which the same mixture of water and milk was given without the addition of chloral hydrate. After waking, the diuresis began to increase. In one of the experiments, in the thirty minute sleep interval preceding waking, a total of 11 ml was secreted from the two kidneys together; in the same time interval, after waking, 44 ml were secreted, i.e., an increase of 4 times.

Another effect could sometimes be observed, depending on the dose. Thus for instance with small doses of chloral hydrate (0.75 g) the diuresis following the milk-water load was higher than in the control experiments.

Finally, it must be pointed out that under the influence of chloral hydrate, in any dose, the rates of excretion of the two kidneys tend to become equalized, so that both under deep sleep induced by either 3 g of chloral hydrate or by morphine, the difference between the excretion rates of the two kidneys is greatly reduced. However when using small doses of chloral hydrate the asymmetry in the performance of the left and right kidneys was well marked.

Thus in experiments on dogs with separately exteriorized ureters, it has been established that in normal conditions there is a difference in the amount and composition of the urine excreted. This functional asymmetry is but weakly shown in the case of spontaneous diuresis, but appears more clearly in the diuresis following a water-milk load and mercuzal. In deep sleep induced by morphine or chloral hydrate, the excretory rates of the two kidneys became more nearly equal. The functional asymmetry in the action of the kidneys depends evidently on the functional condition of the central nervous system.

TABLE

Effect of Morphine on Diuresis (urine collected at 15 minute intervals)

Conditions of experiment	Volume of urine in ml.		Ratio diuresis right kidney/diuresis left kidney, in %.
	Right kidney	Left kidney	
Prior to water-milk load . . .	6 0	5.0	1.20
	7.0	8 0	0.87
After load (600 ml)	12 0	16 0	0.75
2 ml 1% morphine injected subcutaneously *	10.0	10 0	1.00
	14.0	4 0	3.50
	4.0	3.0	1.33
	3.0	3.5	0.86
	3.0	3,0	1.00
	3.0	3,0	1.00
Sleep	1.5	2.0	0.75
	0.5	0.5	1.00
	1.0	1.0	1.00
	1.0	1 0	1.00
	0.5	1.0	0.50
	1.5	1.5	1.00
	1 0	1.5	0.66
Total for 3 hours.	34.0	25.0	1.36

* After the injection of morphine vomiting occurred.

SUMMARY

Experiments on dogs with ureters separately exteriorized have shown that under normal conditions the quantity and quality of urine excreted by each kidney differ.

Functional asymmetry, only weakly marked in spontaneous diuresis is more pronounced in cases of water loading and mercuzal diuresis. Morphine and chloral hydrate narcosis make both kidneys function more evenly. Asymmetry of kidney function is seemingly dependent on the functional state of the central nervous system.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

CHANGES OF RENAL FUNCTION PRODUCED BY HEMISECTION OF THE SPINAL CORD IN DOGS

COMMUNICATION I. THE EFFECT UPON RENAL FUNCTION OF UNILATERAL HEMISECTION OF THE SPINAL CORD AT THE CERVICAL LEVEL

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The homeostatic adjustments of an organism, which manifest themselves after an injury inflicted upon the central nervous system, have been investigated almost solely in the sphere of somatic functions (in the laboratories of F. Holtz, L. Luciani, V. M. Bekhterev, I. P. Pavlov, A. Bete, P. K. Anokhin, E. A. Asratyan and others). The adjustments made in the realm of disturbed vegetative functions have not been really studied even though they are of considerable interest for clinical medicine, especially in the field of neurosurgery and neuropathology. In this field neither the clinician nor the clinical experimenter have much material except what has been picked up accidentally while working on other problems.

The first experiments, having as their deliberate goal the study of the homeostasis of the autonomic system following surgical damage to the central nervous system, were performed by the associates of E. A. Asratyan: in part by Ya. M. Pressman [2] who examined in dogs the role played by the cerebral hemispheres in the adjustments made by the salivary glands, and also by R. O. Barsegyana [1] who examined the effect exerted upon dog gastric functions by the sectioning of the anterior half of the spinal cord. These are the only known experiments studying the homeostatic mechanisms existing within the vegetative system and which make the compensatory adjustments following injury of this or that portion of the central nervous system.

Considering the interest which this problem presents for physiology, as well as for clinical medicine, at the suggestion of E. A. Asratyan, we undertook during the course of 1954-1955 an investigation which sought experimental answers to the questions of how kidney function is disturbed by hemisection of the spinal cord (severing a lateral half); is the function restored in time and what are the dynamics of this restoration, if it occurs? We also had the thought that these studies might shed light on the question of the nervous regulation of kidney function which has so far been a topic of lively discussions.

EXPERIMENTAL METHODS

The studies were conducted on four dogs operated by the Pavlov-Orbeli method whereby the ureters are individually brought out to the surface of the animal's skin with their natural orifices intact.

This method is especially convenient when there is a desire to compare the working of the two kidneys over a long period of time.

After determining the basic functional condition of both kidneys, a right hemisection of the spinal cord was done on dogs: two at the level of the fifth cervical segment, and two others – the first cervical segment.

In our experiments we thus were able to utilize the comparison of the activities of the two kidneys: the kidneys situated on the operated side and the kidneys lying on the opposite side, as our preoperative expectations were that there would be more malfunctioning on the part of the kidney situated on the same side on which the spinal cord hemisection had been performed.

The reason for this expectation lies in the fact that the spinal vegetative centers, homologous with the operated side, lose their closely-knit structural and functional connection with the higher centers as a result of the spinal cord hemisection.

As before, so after the operation the functional state of the kidneys of each operated dog was determined by observing the diuresis, percentage quantity of chlorides, urea and creatinine in 30 minute samples of urine collected from each ureter over experiments lasting 3-6½ hours, under usual kidney states, as well as when functionally burdened with water (400 cc), table salt and urea (10 grams in 150 cc of diluted milk). In addition, the urine was checked qualitatively for albumin.

Chlorides were determined by Moore's method, urea – by decomposition of the alkalinized bromate in the Borodin apparatus, creatinine – by the colorimetric method, employing the concentrating photocolorimeter, while the qualitative test for albumin was performed with the aid of sulfasalicylic acid.

Our experimental animals were under observation from 7½ up to 16 months. The four dogs had a total of 193 experiments performed upon them in the course of which over 5000 biochemical urine analyses were done.

EXPERIMENTAL RESULTS

On two dogs – Kashtan and Belki – the functional state of the two kidneys was studied before and after right-sided spinal cord hemisection at the level of the fifth cervical segment.

Our studies demonstrated that, before surgery, individual differences between the activities of the left and right kidneys were quite insignificant. As an illustration, we append the protocols of two experiments performed on Kashtan and Belki (see Table).

After the right-sided operation, considerable alterations in the activity of the kidney of the same side could be noted. These changes followed definite cyclic intervals.

In the first period (on the 3rd postoperative day) there were no changes in either kidney when compared with their preoperative base level.

It must be admitted that in one dog (Belki) the concentration and amount of chlorides produced by the kidney of the operated side was somewhat depressed.

In the second period (from the 7th to 22nd-28th postoperative days) the kidney of the operated side developed an increasing diuresis. The kidney on the operated side during the time of the experiment (4-5 hours) produced from 10-40% more urine than the opposite kidney and as compared with its preoperative function. Some individual 30 minute samples from the right kidney produce twice the amount put out by the left.

Along with this diuresis, the urine from the kidney of the operated side showed a diminution in its content of urea and ceatinine. Along with this the concentration of the chlorides rose sharply in Kashtan (sometimes 2-4 fold) while remaining depressed in dog Belki.

In the third period (in Kashtan, from 22nd to 47th, in Belki from 28th to 70th days) urinary output from the right side diminished to only a half of that from the left, in some samples to one-third.

Associated with this there was noted a marked rise in the concentration of the urea, creatinine and chlorides in urine samples from this kidney (2-4 fold) as compared with the concentrations of the same substances from the left kidney.

In the fourth period, in Kashtan beginning with 47th day, in Belki from 70th day, the relationships of these indicators reverted to their normal state.

TABLE

Diuresis, Urea, Chlorides and Creatinine Before and After Right Sided Hemisection of the Spinal Cord at the Fifth Cervical Segmental Level in Dogs Kashtan and Belki

30 min. sample	diuresis (in cc)			urea (in %)			chlorides (in %)			creatine (in %)		
	left kidney	right kidney	correlation	left kidney	right kidney	correlation	left kidney	right kidney	correlation	left kidney	right kidney	correlation
Kashtan. Before surgery. Exp. No. 14 March 26, 1954												
1	7.0	6.5	100 : 93	1.34	1.34	100 : 100	0.04	0.04	100 : 100	0.050	0.051	100 : 101
	Table salt given											
2	13.0	12.5	100 : 96	3,35	3,3	100 : 98	1.09	1.18	100 : 101	0.147	0.152	100 : 103
3	26.5	25.5	100 : 96				1.77	1.8	100 : 101			
4	54.5	54.0	100 : 99				1.8	1.83	100 : 101			
5	68.0	66.0	100 : 97	0,22	0,22	100 : 100	1.8	1.83	100 : 101			
6	34.0	33.5	100 : 98				1.97	1.98	100 : 100	0.008	0 008	100 : 100
7	22.5	21.0	100 : 93				1.9	1.9	100 : 100			
8	15.0	15.0	100 : 100				1.99	1.88	100 : 99			
9	9.0	8.5	100 : 94	0,77	0,7	100 : 91	2.07	2.11	100 : 102	0.034	0.035	100 : 100
7th day after operation. Exp. No. 27, May 3, 1954												
1	3.7	3.0	100 : 81	3.052	2.051	100 : 67	0.092	0.350	100 : 380	0.101	0.076	100 : 75
	Water given											
2	3.6	3.5	100 : 97	4.197	3 052	100 : 72	0.058	0.174	100 : 300	0.116	0.081	100 : 69
3	35.5	35.0	100 : 98									
4	62.0	60.5	100 : 97	0.477	0.381	100 : 80	0.022	0.038	100 : 172	0.0062	0.006	100 : 96
5	47.0	50.0	100 : 106									
6	11.0	15.0	100 : 136									
7	4.0	5.3	100 : 133	1.908	1 526	100 : 80	0.058	0.122	100 : 210	0.077	0.06	100 : 78

TABLE (continued)

30 min. sample	diuresis (in cc)			urea (in %)			chlorides (in %)			creatine (in %)		
	left kidney	right kidney	correlation	left kidney	right kidney	correlation	left kidney	right kidney	correlation	left kidney	right kidney	correlation
					26th postoperative day. Exp. No. 35, May 22, 1954							
1	6.0	2.2	100 : 36	2.926	7.457	100 : 255	0.339	1.426	100 : 420	0.065	0.162	100 : 249
	Table salt given											
2	6.0	2.2	100 : 36	2.501	6.985	100 : 279	0.467	1.648	100 : 353	0.063	0 176	100 : 279
3	8.0	6.1	100 : 76				0.613	1.706	100 : 278			
4	40.0	28.7	100 : 71				1.777	2.461	100 : 138			
5	59.5	33.0	100 : 55	0.424	0.802	100 : 189	1.66	2.379	100 : 143	0.007	0.013	100 : 185
6	44.5	23.2	100 : 52				1.666	2.583	100 : 155			
7	35.5	19.0	100 : 53				1.665	2.624	100 : 157			
8	25.0	14.0	100 : 56				1.638	2.718	100 : 166			
9	20.5	11.5	100 : 56	0.66	1.321	100 : 200	1.59	2 776	100 : 174	0.015	0.034	100 : 226
					Belki. Before surgery. Exp.No.16 March 26,1954							
1	3.0	3.0	100 : 100	3.54	3.35	100 : 95	0.08	0.08	100 : 100	0.129	0.116	100 : 89
				Table salt given								
2	4.0	4.0	100 : 100				0.33	0.33	100 : 100			
3	5.0	5.0	100 : 100	2.15	1.77	100 : 82	1.71	1.64	100 : 96	0.068	0 052	100 : 76
4	27.5	27.5	100 : 100	0.62	0.48	100 : 77	1.84	1.75	100 : 85	0.017	0.015	100 : 88
5	28.0	28.0	100 : 100				1.87	1.8	100 : 96			
6	17.5	17.0	100 : 97				1.94	1.89	100 : 97			
7	11.0	11.0	100 : 100				1.99	1.91	100 : 96			
8	7.5	7.5	100 : 100				2.07	1.94	100 : 94			
9	7.0	6.5	100 : 93				1.98	1 87	100 : 94			
10	4.5	4.0	100 : 89	1.15	1.1	100 : 96	2.05	1.94	100 : 94	0.059	0.051	100: 86

TABLE (continued)

30 min. sample	diuresis (in cc)			urea (in %)			chlorides (in %)			creatine (in %)		
	left kidney	right kidney	correlation	left kidney	right kidney	correlation	left kidney	right kidney	correlation	left kidney	right kidney	correlation
19th postoperative day. Exp. No. 34 May 15,1954												
1	2.1	2.5	100 : 119	6.72	5.184	100 : 77	1.034	0.596	100 : 58	0.152	0 096	100 : 62
Table salt given												
2	2.2	2.8	100 : 126	6.144	4.608	100 : 75	1.479	0.847	100 : 57	0.148	0.098	100 : 67
3	4.8	5.5	100 : 115				2.835	2.145	100 : 76			
4	30.5	41.0	100 : 134				2.332	1.964	100 : 72			
5	32.0	57.0	100 : 178	0.72	0.24	100 : 34	2.279	1.853	100 : 81	0.011	0.006	100 : 54
6	29.6	34.5	100 : 116				2.239	2.016	100 : 90			
7	21.0	35.0	100 : 166				2.502	2.046	100 : 82			
8	25.0	28.0	100 : 112				2.32	2.005	100 : 86			
9	15.5	19 5	100 : 126				2.331	2.057	100 : 88			
10	13.5	15.0	100 : 111	1.344	0.432	100 : 32	2.519	2.104	100 : 83	0.026	0.018	100 : 69
28th postoperative day. Exp.No.34 May 15,1954.												
1	5.7	2.7	100 : 47	3.414	6.638	100 : 192	0.859	1.432	100 : 166	0.156	0.253	100 : 162
Water given												
2	9.5	5.0	100 : 52	2.987	5.263	100 : 175	1.23	1.853	100 : 150	0.093	0.131	100 : 140
3	37.3	27.0	100 : 72									
4	56.7	47.5	100 : 83	0.367	0.486	100 : 132	0.277	0.295	100 : 107	0.011	0.013	100 : 118
5	28.0	19.0	100 : 67									
6	15.7	9.2	100 : 58									
7	13.2	7.2	100 : 54									
8	12.0	6.1	100 : 51	2.086	3.746	100 : 179	0.774	1.131	100 : 146	0.056	0.090	100 : 160

Kashtan, in addition, had a fifth period (from 52nd to 73rd day) characterized by a secondary diuresis accompanied by correspondingly diminished concentrations of urea, creatinine and chlorides in the kidney on the operated side. Only after that was their return to base level in the kidney functions studied.

The basic data is presented in the table.

We also examined this problem from the standpoint of spinal cord hemisection well above the spinal sympathetic centers, i.e., at the level of the first cervical segment. This experiment was conducted also on two dogs — Zolushke and Rzhei.

Essentially, the changes observed were similar to those seen in the fifth cervical sement hemisections except that they were less pronounced and characterized by certain peculiarities. In part, the kidney on the operated side failed to develop a diuretic phase accompanied by increased concentrations of urea, creatinine and chlorides which was a characteristic of the dogs who underwent surgery at the fifth cervical segment level.

Judging by our accumulated data, in all the operated dogs kidney functions were fully back to normal within $2\frac{1}{2}$-3 months after the hemisections of either the higher or lower cervical spinal cord segments. Somatic functions—standing, walking and running— returned in these same animals within 35-40 days, i.e., 2 times more quickly than the kidney functions.

In regard to the albumin which was determined qualitatively, the following was noted. After right-sided hemisection at the fifth cervical segment, both kidneys developed traces of albumin, the right in a slightly greater amount. Within 7 days the albumin was gone from the left-sided specimens although still present at 7-10 days in samples obtained from the right side. In dogs subjected to hemisection at the level of the first cervical segment, albumin appeared only in specimens from the operated side and then was gone within the week.

SUMMARY

Four dogs with individual fistulae of each ureter operated by the Pavlov-Orbeli method were studied. Before and after hemisection of the spinal cord at the level of the cervical segments, diuresis, urea, chlorides and creatinine concentration have been estimated in the urine of each kidney separately on the normal and operated side. Hemisection of the spinal cord at the level of the first or fifth cervical segment caused phasic changes in the function of the kidney on the operated side, the more so when hemisection was performed at the level of the fifth segment. All tests performed indicated that disturbed renal function is fully restored in the course of 2.5-3 months, while somatic functions of the same dogs were fully restored in 35-40 days.

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ACTION POTENTIALS OF RESPIRATORY MUSCULATURE IN PATIENTS HAVING RESPIRATORY DEFICIENCY

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Many important investigations, devoted to an experimental analysis of various phases of the activity of the respiratory system in animals, have fulfilled their goal with the aid of the electrophysiological techniques.

At the same time, this electrophysiologic method has been used but rarely in the study of respiration in man. Only quite recently have there appeared investigations devoted chiefly to the way in which the anterior abdominal muscles along with certain others participate in the respiratory act [3, 4, 5, 6]. It has been discovered that action potentials develop in abdominal muscles only in association with deep breathing and is connected with arise in intra-abdominal pressure. E. L. Golubova [1] was able to record the action potentials of the rib muscles in human individuals undergoing lung resection and thus observe whether regulation of breathing led to any peculiarities. These characteristics have been studied in great detail by her when she removed experimentally lungs in animals.

In proceeding with the present research we used as a basis preceding studies [2] which indicate that in a healthy man artificial interference with respiration sharply raises the bioelectric activity of the respiratory musculature.

In rabbits when resistance to respiration is increased the action potentials of the respiratory muscles increase gradually mainly by humoral stimulation. In man, however, there is immediate reflex response to the increased resistance and there does not appear to be any evidence of a humoral mechanism. This gave us ground for supposing that an analogous increase in action potentials would be detected in certain diseases of the respiratory system as, for example, pulmonary emphysema and bronchial asthma in which condition there is a present a resistance to the flow of air.

EXPERIMENTAL METHODS

We studied 10 persons who were well and 65 patients having respiratory inadequacies (principally patients with bronchial asthma and pulmonary emphysema). In order to record the action potentials of the respiratory musculature, electrodes were applied to the overlying skin. One pair of electrodes was fastened in the anterior axillary line between the seventh and eighth ribs, the other pair — on the skin over the abdominal wall overlying the external oblique muscle. The action potentials were recorded on photographic paper by means of a magnifying oscillograph built by the Leningrad Technological Institute. The time signal and the pneumogram were recorded simultaneously on the photopaper.

EXPERIMENTAL RESULTS

When breathing quietly (sitting) action potentials in normal people with the amplification we used (100 μV corresponded to a deflection by the beam of 5 mm) were almost unnoticeable or represented very weak salvos

of impulses during the inspiratory phase on the part of the intercostal musculature and was totally absent from the external abdominal musculature (Fig. 1).

In patients suffering from moderately severe bronchial asthma and in patients having pronounced pulmonary emphysema, the action potentials have a different character. In patients suffering from bronchial asthma there is observed an almost constant, markedly expressed activity by the intercostal muscles, reaching a maximum

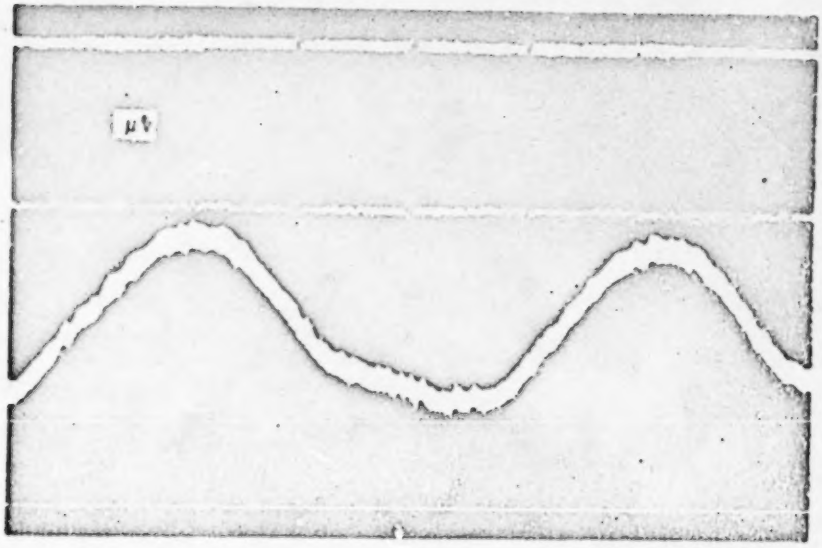

Fig. 1. Action Potentials in the respiratory musculature during quiet breathing of a healthy individual.
1) Electromyogram of intercostal muscles; 2) electromyogram of the external oblique of the abdomen; 3) pneumogram; 4) time markers (1 second). In this and the other figures there can be noticed the overlay of the electrocardiogram on the electromyogram.

during prolonged expiration; during this expiration there being noticed also activity on the part of the abdominal muscles (Fig. 2). Thus, the electromyograms document the action during expiration which in a healthy individual is a passive act thus permitting the observation of increased respiratory activity even when it appeared from the outside to be of almost normal rate and depth.

Patients suffering from pulmonary emphysema also show a marked increase in the action by the respiratory musculature. The lead from the intercostal musculature shows continuous activity throughout the entire respiratory cycle (Fig. 3), being most evident during inspiration; the action potentials, even if of much lesser amplitude, can be noticed also on the electromyograms of the abdominal musculature.

Thus, it is evident that electrophysiologic observations on patients suffering from respiratory inadequacies demonstrate that even when quiet there is compensatory increase in the activity of the respiratory musculature, this being an attempt to ensure the necessary ventilation of the lungs.

The electrophysiologic method for the study of the functioning of the respiratory system permits direct observation of the respiratory musculature and of the nature of the stimulation acting on the respiratory center. When doing research on the breathing of a normal individual, the degree to which the respiratory center is being stimulated is taken to be a function of pulmonary ventilation. Actually, however, this is really an end effect stimulating the respiratory center.

Of course, the nature of the impulses going from the respiratory center over the motor neurons to the respiratory musculature determines the character of the respiratory movements and therefore the depth and the

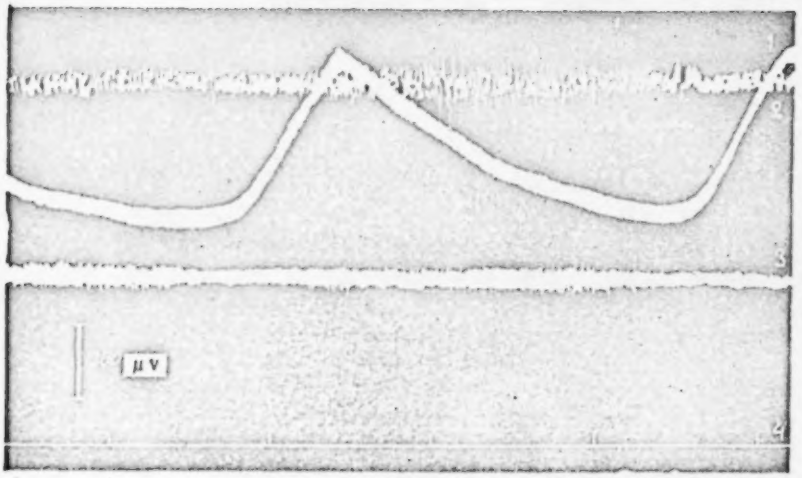

Fig. 2. Action potentials in the respiratory musculature during quiet breathing of a patient suffering from moderately severe asthma.
1) Electromyogram of intercostal muscles; 2) electromyogram of the external oblique of the abdomen; 3) pneumogram; 4) time markers (1 second). In this and the other figures there can be noticed the overlay of the electrocardiogram on the electromyogram.

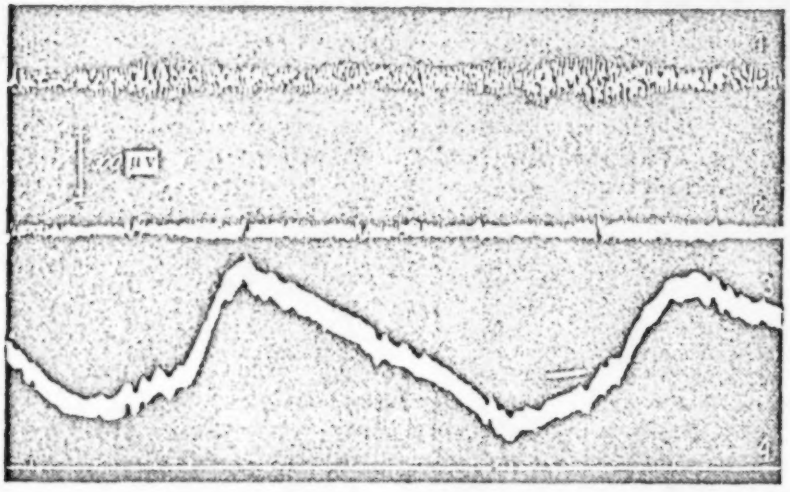

Fig. 3. Action potentials in the respiratory musculature during quiet breathing of a patient with pulmonary emphysema.
1) Electromyogram of intercostal muscles; 2) electromyogram of the external oblique of the abdomen; 3) pneumogram; 4) time markers (1 second). In this and the other figures there can be noticed the overlay of the electrocardiogram on the electromyogram.

respiratory rate which determine the magnitude of the lung ventilation. Still, this can be true only if there is a direct relation between the stimulus arising in the respiratory musculature and the resulting activity-pulmonary ventilation. But in many instances, when the pulmonary ventilation is upset by external causes or pathological processes, this normal relationship cannot be maintained.

In such instances direct electrophysiological studies and not simply observation of the pulmonary ventilation give us the possibility of judging truly the nature of the impulses going from the respiratory center to the respiratory muscles, i.e., the true condition of the respiratory center. For this reason the study of action potentials in patients having respiratory malfunction offers important evidence of the true condition of respiratory regulation.

Many of the patients we observed had normal pulmonary ventilation breathing rate and depth. Yet the clinical picture of dyspnea pointed to respiratory inadequacy.

In such cases, as demonstrated by the marked increase in action potentials, the pulmonary ventilation was made to approach normal by a marked increase in the activity of the respiratory musculature, this being attained by a constant over-stimulation of the respiratory center. This seems to be the principal compensatory reaction without which there would be no guarantee of an adequate pulmonary ventilation under conditions of interference with the apparatus for external respiration.

It may be supposed that the continued over-activity of the respiratory system produces frequently alterations in the state of the respiratory center and its cerebral radiations which express themselves as oppressive sensations (dyspnea, in the beginning), decreased work capacity and other symptoms. The action potentials in many patients at rest are as great as in normal people performing heavy physical labor.

It can thus be seen that this method of evaluating the action potentials present in the respiratory musculature, along with other methods, gives objective aid in clarifying many important problems of respiratory physiology as well as pathology in man, aids in uncovering the qualitative characteristics and the mechanisms producing those clinical states presenting a picture of respiratory inadequacy.

SUMMARY

Normal action potentials in respiratory muscles were studied. Then these were compared with action potentials observed in patients, chiefly those ill with bronchial asthma and pulmonary emphysema. It was noted that the muscles of the patients registered a marked rise in activity even when the usual external indicators such as rate, depth and pulmonary ventilation appeared to be near normal. This was taken to indicate that the respiratory center was being subjected to compensatory stimulation.

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ALTERATIONS OF HEMODYNAMIC INDICATORS AND OF THE ELECTROCARDIOGRAM PRODUCED BY EXPERIMENTAL DISTURBANCE OF THE CORONARY CIRCULATION

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The pathology of the coronary circulation has still not been exhaustively studied in extensive experiment. Some investigators in this field [4, 6, 7, 8] have studied the details of the collateral circulation within the myocardium but have not explored adequately the functional alterations produced in the coronary circulation by disturbances affecting the blood supply of the myocardium. The foreign literature also is silent on hemodynamic alterations produced in the coronary circulation by means of experimentally introduced pathology. Yet in order to develop experimentally the therapy for myocardial infarction and coronary insufficiency it is essential to have precise knowledge of those hemodynamic alterations which occur in model coronary insufficiency. Besides the theoretical significance, facts of this type have a definite interest in refining some of our concepts as to the symptomatology of myocardial infarction. This communication is devoted to an examination of the experimental material obtained in dogs who were made, artificially, to develop an insufficiency of the myocardial circulation.

EXPERIMENTAL METHODS

The experiments were conducted on adult dogs of both sexes and of varying weights. Coronary insufficiency was produced by applying a ligature to the descending branch of the left coronary artery at the juncture of its middle and lower thirds or else to the left coronary artery in the region of its bifurcation. Aseptic procedures were employed. The heart was approached by an incision below the fourth rib. Special spreaders were used to enlarge the opening. After the pleural cavity had been opened, artificial respiration was employed. The experimental dogs were followed for 40 days after surgery. At that time they were sacrificed and their hearts subjected to a searching pathological anatomical study. The electrocardiogram registered alterations in the actions of the coronary system; also, alterations in blood pressure, pulse and velocity of blood flow were recorded. The EKG was recorded in the three standard and two chest leads (V_5, V_1); the chest electrode in V_5 tracing was applied in the fifth interspace in the anterior axillary line, in the V_1 lead – in the fourth space just to the right of the sternum. The maximum blood pressure was recorded from the left carotid artery which had been previously brought out in a skin pouch. Velocity of blood flow was determined by the lobeline method.

EXPERIMENTAL RESULTS

a) EKG changes and hemodynamic alterations produced by ligation of the descending branch of the left coronary artery at the juncture of its middle and lower thirds.

The EKG picture produced by this ligation is uniform and is, basically, a change in T and the S–T interval. Table 1 summarizes the characteristic EKG changes.

The maximal blood pressure changes occurred in the acute state of myocardial ischemia (the first five days after operation) and went through two phases: on the day of ligation the blood pressure in two instances was markedly elevated, being unchanged in two others. Then, by the second day the blood pressure fell below base level remaining elevated in only one experiment, dog Blackie). Thus, for example, dog Cherny whose normal pressure was steady at 134-137 mm mercury pressure, the pressure immediately after the operation rose to 160 mm, then dropped sharply on the second day and remained within the limits of 124-136 mm.

TABLE 1

The Nature of the EKG Changes After Ligation of the Descending Branch of the Left Coronary at the Juncture of its Middle and Lower Thirds.

Dog	EKG Changes			Remarks
	Day of ligation	5 days after ligation	After 40 days	
Lex	P(2,3) enlarged T + (2, 3) Q−T enlarged T −(1) extrasystole	T + (2, 3, V_2, V_1) S−T + V_5, V_1)	Normalization	T normally is absent in (1), in others leads −
Cherny	T diminishes (2, 3, V_5, V_1) S−T + + (2, 3, V_5, V_1), extrasystole, alternating pulse	T diminishes (2, 3), Q−(2, V_5) lowered voltage	Q−(2), S−T + (2), T diminishes (2, 3, V_5), lowered voltage	
Lisa	T depressed (2, V_5), S−T + (2, V_5), P enlarged (2, 3, V_5), lowered voltage	S−T + (V_5, V_1), T + (V_5, V_1), T absent in other leads	S−T + (2, 3, V_5), T normalized voltage increased	T normal − (1, 2, 3, V_5) and + (V_1)
Zhelty	T (1, 2, 3), Q−T prolonged, extrasystoles, alternating pulse	S−T + (2, 3), T + (2, 3), depressed voltage	T−, diminished (2, 3), S−T + Q−(2, 3)	T is normal
Pesets	Polymorphic extrasystoles, tachycardia	S−T + (2, V, V), T enlarged (2, 3), diminished (V_5, V_1), lowered voltage (V_5)	S−T + (2, V_5, V_1), T depressed (V_5)	

Remarks. Here and in Table 2 the plus sign indicates relation of T wave and S−T interval to the isoelectric line. The numbers in brackets indicate the leads of EKG.

The alterations in the frequency of the heart beats had a converse character: on the day of operation the rate fell markedly (Zhelty −from 118 per minute to 58) and only in one case (Lex) there was observed a slight rise (from 88 to 104), while even here by the 4th day after ligation there was a pronounced slowing of the rhythm (to 54). In the other experiments, after the immediate postsurgical slowing, the pulse gradually returned to the preligation levels. By the end of the period of observation (40 days) there were no material deviations from the normal.

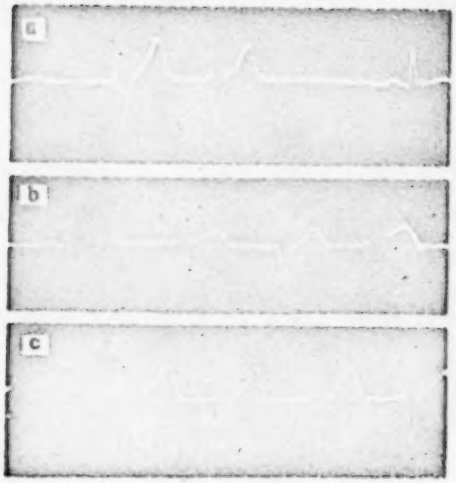

Fig. 1. Changes of EKG after ligation of the descending branch of left coronary artery at the juncture of middle and lower thirds (dog Cherny, third day after applying ligature). a) Lead II, b) lead III, c) lead V_6.

TABLE 2

Characteristic EKG Changes Occurring After Ligation of the Descending Branch of the Left Coronary Artery in the Region of its Origin at the Bifurcation

Dog	EKG Changes			Remarks
	Days of ligation	5 days after ligation	After 40 days	
Bat	T − (1, V_6), QRS shortened, S−T + (1, V_6), polymorphic extra systoles, pulsus alternans	T + Q (3) vanished, lowered voltage, paroxysmal tachycardia, extrasystoles	T − (1), Q vanished (3), P−Q prolonged	T normal +
Gek	T + (1, 2, 3), diminished voltage (V_6, V_1)	T increased (V_6, V_1), S−T + (V_6, V_1)	T diminished, S−T + (V_6, V_1), P−Q prolonged	T normal − (2, 3) and + + V_6, V)
Chuk	T enlarged (2, V_1), S−T + (V_1). Polymorphic extrasystoles, pulsus alternans	T diminished, P−Q enlarged voltage lowered	T−(2), P vanished (3) Q−T diminished, lowered voltage, atrioventricular block	T normal +

Thus, it can be seen that changes, following ligation of the descending branch of the left coronary artery in its lower third, in so far as the blood pressure is concerned seem to be of very short duration. This indicates rapid compensation of the myocardial disturbance caused by the myocardial ischemia. The rise of the blood pressure on the day of surgery must be related to the reconstruction of the hemodynamics necessitated by the pneumothorax which, as has been shown experimentally [1, 3], is accompanied by sharp rises in blood pressure, and also related to a compensatory blood pressure rise observed to occur as a result of myocardial ischemia [2]. In cases when this reaction did not occur postoperatively (Lisa, Zhelty), there took place a severe fall in blood pressures later which, in all probability, was associated with weak compensatory mechanisms in these animals. The bradycardia observed in the acute ischemic phase must be regarded as a consequence of the increased tone on the part of the recurrent nerves because of the rise in the strength of the impulses emanating from the receptors in the vicinity of the infarcted myocardium and the adjacent pleura.

The clearest and most constant indicators of the instability and insufficiency of the coronary circulation resulting from the ligation are seen on the EKG. The changes and evolution of the T wave and S–T segment are characteristic of infarcts of the anterior wall of the left ventricle. As a rule, this ischemia activates the secondary automatic centers and this manifests itself as concordant extrasystoles (Fig. 1).

However, the circulatory changes produced by ligation of the descending branch of the left coronary at the juncture of the middle and lower thirds of the artery are rapidly compensated by the collateral circulation which, along with the data on the functional state of the cardiovascular system, confirms also the autopsy data which fails to reveal any wide-spread necrotic alterations in the myocardium.

b) EKG changes and hemodynamic alterations produced by ligating the descending branch of the left coronary artery near its origin at the bifurcation.

Ligation of the descending branch of the left coronary artery near its origin at the bifurcation produces the EKG pattern characteristic for an anterior wall infarct: inversion of T, S–T segment elevation, sensitivity alterations typical for this localization of the infarct and leading to characteristic EKG evolution (Table 2). With the higher ligation of the descending branch, the hypotensive swing from the maximal blood pressure was more pronounced so that, even on the day of ligation, there was noted a marked fall in blood pressure. Thus in dog Chuk the maximal blood pressure fluctuated normally between 108-126 mm mercury, while, after ligation, it fell to 84 mm and for five postoperative days did not exceed 88 mm. In all the experiments of this series there developed a tachycardia relative to the normal base. Dog Gek before surgery had a pulse of 66-95 per minute, while after the ligation the rate varied from 96 to 114 per minute, the similar data for Chuk being a rise from a pulse of 108 up to 180. Over the entire period of observation following the ligation there was also noted a rise in the circulation rate (Table 3).

TABLE 3

Changes in Blood Velocity After Ligation of the Descending Branch of the Left Coronary Artery

Dog	Blood velocity (in seconds)	
	Normal	After applying ligature
Chuk	11.5–12.6	8.5– 9.1
Gek	11.0–12.0	9.0–11.2

These marked alterations in cardiac contractions and blood velocities bespeak a mobilization of the compensating mechanisms existing in the cardiovascular system.

The gross and microscopic evidence of the results of the autopsies performed on the sacrificed animals confirms the presence of extensive anterior apical infarcts (Fig. 2).

However, it must be noted that pronounced insufficiencies of the cardiovascular system did not develop even when the descending branch of the left coronary artery was ligated near the bifurcation.

In order to clarify the role played by the recurrent branch of the vagus in the developing complex of changes, we conducted a series of experiments in which atropine was employed. Injecting atropine into dogs regularly having extrasystoles led, as a rule, either to abolition of these extrasystoles altogether or else to a

Fig. 2. Manifestations of scarring near the tip of heart in the ischemic zone. Inner surface of left ventricle (dog Chuk).

marked diminution in their number (Fig. 3). However, in these instances of rhythm normalization there were, sometimes observed marked EKG abnormalities. Our findings indicate that activization of foci of heterotopic excitation is associated with influences transmitted by the recurrent nerves. This coincides with the results

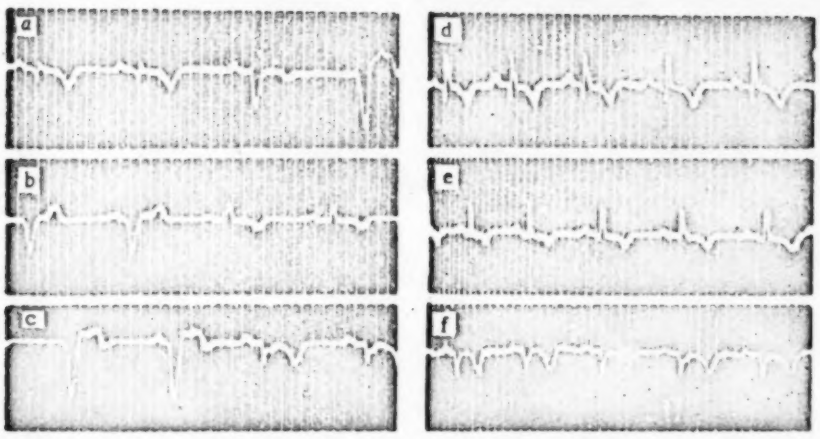

Fig. 3. The influence of atropine upon the rhythmic acitivy of the heart. I) EKG before atropine, a–lead II, b–lead III, c–V_6; II) EKG 15 minutes after internal administration of atropine (labeled as in Fig. 1).

obtained by A. I. Smirnov and A. I. Shumilova [5] who demonstrated that either section of the recurrent nerves or the use of atropine eliminated extrasystoles present when experimental myocardial infarcts were produced. The absence of undesirable hemodynamic changes in the course of excluding pharmacologically the recurrent nerve gives us the possibility of recommending atropine as a drug to be employed when extrasystoles develop clinically as a result of myocardial infarction.

SUMMARY

Dogs were used to create infarcts of the anterior myocardial wall. The descending branch of the left coronary artery was ligated either near its origin or else at juncture of middle and distal thirds. The animals were observed for 40 days postoperatively and then sacrificed for complete cardiac studies. The EKG, the B.P. and heart rate were taken throughout this experimental series.

Atropine was used to abolish extrasystoles. The hemodynamic alterations were transient while the EKG ran the course usual for this type of infarct.

It is suggested that the recurrent nerve mediates heterotopic foci of cardiac excitation.

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INTEROCEPTIVE REFLEXES OF DOGS DURING HYPOGLYCEMIA AND SUBSEQUENT RESTORATION OF THE BLOOD SUGAR LEVEL

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In short experiments on cats we have shown [7] that, as hypoglycemia progressively develops, it is constantly accompanied by phasic alterations in the pressor interoceptive reflexes.

The present research was undertaken to confirm the changes taking place in the absence of narcosis as well as to exclude the initial hyperglycemic stage inevitable in a short experiment.

EXPERIMENTAL METHODS

Six dogs were used in our experiments (3 females and 3 males). All the animals had gastric fistulae and in three one carotid artery was exteriorized. Blood pressure changes were followed as the mechanical receptors of the stomach were stimulated as well as those in rectum by use of methods we described previously [9], all this being followed by observing the sino-carotid pressor reflex. This last was induced by compressing with a finger for 30 seconds the carotid artery lying in the skin tag.

Insulin was given intravenously in increasing dosage in the experiments as they were repeated.

To terminate the hypoglycemic state intravenous glucose was employed as well as subcutaneous or intravenous adrenalin.

Blood sugar was determined by the Hagedorn-Jansen method.

Altogether there were 48 experiments: 34 to investigate the interoceptive reflexes under condition of hypoglycemia, 9 control and 5 for method.

Five hundred and sixty blood sugar determinations were made.

The general experimental conditions, insulin dosage and the degree of hypoglycemia attained are presented in the table.

EXPERIMENTAL RESULTS

As in our preceding short experiments on cats, in the first phase of hypoglycemia we observed heightening of the pressor interoceptive reflexes with a lowering of reactive threshold (Fig. 1, tracings 4-6). Later the reflexes became depressed or disappeared entirely (Fig. 1, tracings 7-9), while in an entire series of experiments, instead of pressor reflexes, a depressor reaction appeared.

As the animal emerged from the hypoglycemic state, the interoceptive reflexes were restored going, in many instances, through a phase of heightened pressor responses (Fig. 1, tracings 10, 11) which confirms the fact of the two phasic changes undergone by the interoceptive reflexes when the blood sugar level is reduced.

TABLE

Dogs	Wt (in kgs.)	No. experiments	Amount 40% Glucose before Insulin (in cc)	Quantity insulin		Blood sugar (in mg %)					Length of observation after giving insulin
				Units per kg.	cc.	Base	Record of background	Minimal level after insulin	Decrease (in mg %) from base level	Decrease (in mg %) from maximum after glucose	
Basic experiments											
Grad	17.9—21 6	8	—	2—8	0.9—4.0	98—113	96—120	58—38	53—68		1 hour 29 min. —3 hour
Druzhok . . .	12.8—14.9	7	—	2—11	0.7—3.9	85—105	83—113	56—38	40—60		2 hour 07 " —3 " 33 min.
Zhuchka . . .	15.5—16.3	5	—	1.5—12	0.6—4.9	95—98	93—107	53—41	52—62		1 hour —3 " 25 "
Dirka	22.6—24.6	4	—	2—9	1.2—3 4	83—94	85—94	49—41	38—45		1 hour 26 min —3 " 49 "
Duglas	31.5—32.1	3	—	1.5—5	1.2—4.0	101	98—106	52—43	46—58		2 hour 14 " —3 " 53 "
Dzhanka . . .	19.9—20.9	3	—	2—4	1.0—2.0	89—97	82—114	50—48	34—66		1 hour 26 " —2 " 37 "
Experiments with preliminary glucose											
Grad	21.0	1	60	12	6.3	95	193—348	54	67	294	2 hours 54 min
Druzhok . . .	14 1	1	58	16	5.0	92	194—454	37	59	395	3 " 07 "
Dzhanka . . .	20.0—20.9	2	40—56	8—10	4.0—5.2	95—101	212—358	50—41	53—62	298—308	2 " 29 " —3 hours

In this manner the present studies confirm the results we obtained in our short experiments investigating the character of the changes undergone by the interoceptive reflexes under hypoglycemic conditions.

Nevertheless, the comparison of the short experiments with the series brings out one clear difference: whereas in the acute experiments heightened pressor reflexes predominated, in the present series studies the phase of reflex depression or actual suppression was much the more pronounced.

An explanation of this difference can be sought in special experiments in which the dogs received a preliminary injection of glucose, this being done because in the acute cat experiments (under condition of narcosis) the insulin was being injected against a background of hyperglycemia (up to 354 mg%).

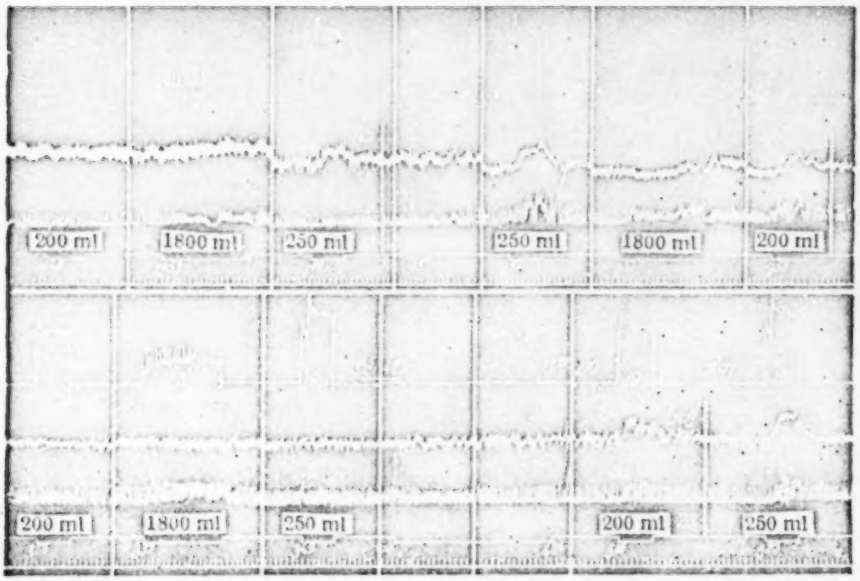

Fig. 1. Two phasic changes in the interoceptive reflexes and thresholds of stimulation as hypoglycemia developed from intravenous injection of insulin (2 units/kg) and then was followed by introduction of glucose. Dog Grad, experiment April 11, 1956. Reflexes from rectum – 1, 3, 4, 6, 7, 9, 10, 11; reflexes from stomach – 2, 5, 8. Significance of tracings (from above down): Pressure within organ, blood pressure, respiration, stimulation marker, time signal (5 seconds). Insulin ↓, glucose ↑.

As can be seen from Fig. 2, when the dogs received preliminary doses of glucose sufficient to maintain for 53 minutes the blood sugar at levels of 200-330 mg %, there was observed a pronounced phase of heightened pressor reflexes which continued for three hours, this being something never observed when insulin was injected against a background of normal blood sugar levels.

In this experiment we never were able to obtain the phase of depressed pressor reflexes even when the lowest glycemic level was 42 mg% which is somewhat below that in the usual experiments (48 mg%).

The introduction of glucose after the insulin, "removed" in this experiment the heightened pressor reflexes and also normalized the general behavior of the animal.

Thus we believe that we can conclude that the differences in the first and second stages between the short and series experiments seen after the injection of insulin are explained by a difference in the basic glycemic level which, in turn, determines the length of time the blood sugar remains depressed initially.

A second reason for this difference lies in the different duration of the period of low glycemic levels.

Under narcosis there were no hypglycemic convulsions and the second hypoglycemic phase in the cats progressed rapidly to the death of the animal. In the dogs, the convulsions which developed led to an immediate rise of the blood sugar level, maintaining it at a low but safe level.

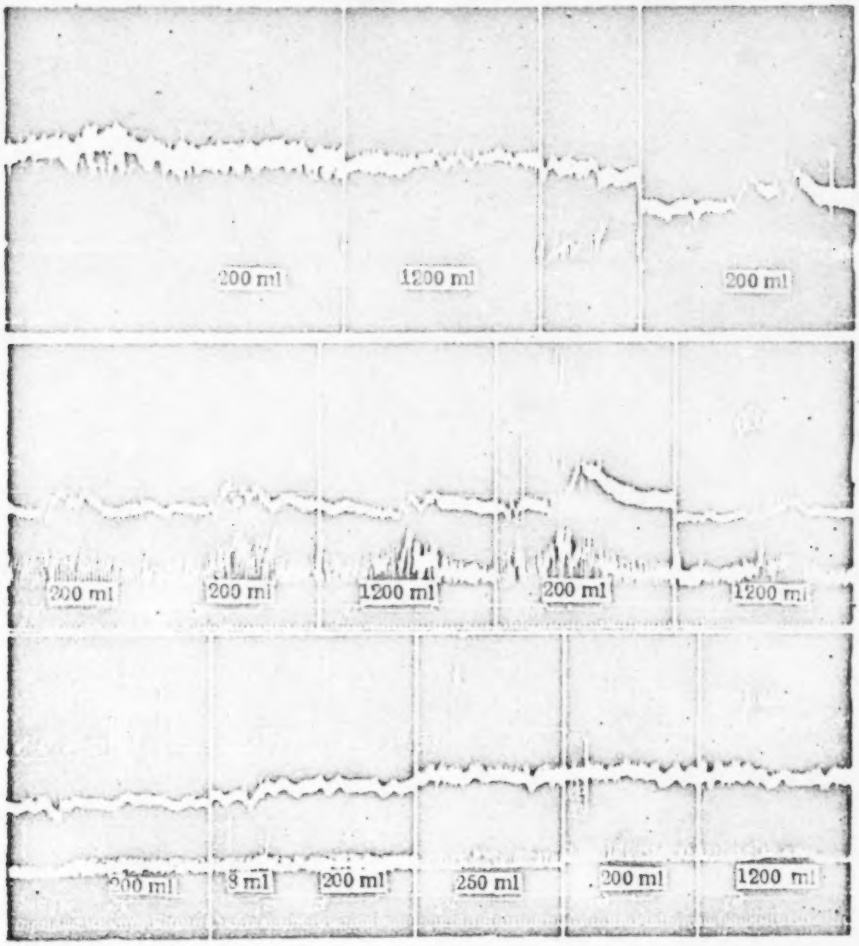

Fig. 2. Alterations in the interoceptive reflexes following injection of insulin (10 units/kg) against a background of hyperglycemia. Dzhanka, Experiment November 25, 1955. Before insulin, glucose was introduced thrice intravenously in the amount of 56 cc of a 40% solution. Reflexes from rectum—1, 3, 4, 5, 7, 9, 10, 11, 12; reflexes from stomach— 2, 6, 8, 13. Significance of tracings same as in Fig. 1.

Thus, in dogs there was a more rapid drop in blood sugar levels after insulin was injected, these precomatose levels being maintained for a longer time, this determining the rapid transition to the phase of depressed interoceptive reflexes as well as the predominance of this phase of the experiments conducted without anesthesia.

In the preconvulsive, as well as in the postconvulsive, periods the pressor interoceptive reflexes were suppressed.

At low glycemic levels we observed the inversion of pressor reflexes into depressive reactions, this being true of the mechanical receptors of the stomach and rectum as well as the sino-carotid reflex. This last was seen against a background of a weakened or absent pressor sino-carotid reflex after restoration of blood flow in the carotid artery (Fig. 3, tracings 8 and 9).

There are grounds for the supposition that this peculiarity of the sino-carotid reflex, observed in a period of diminished or absent pressor interoceptive reflexes, is a consequence of decreased irritability of the vasomotor center accompanied by an increase of inhibitory influences upon it.

The described alterations in the interoceptive reflexes were not observed in control experiments.

As an additional control, we introduced glucose in every experiment in which the blood glucose level had been lowered. This fully confirmed the findings in our short experiments [8] as to the normalizing effect of glucose both in the first hypoglycemic phase as well as in the second (Figs. 1-3).

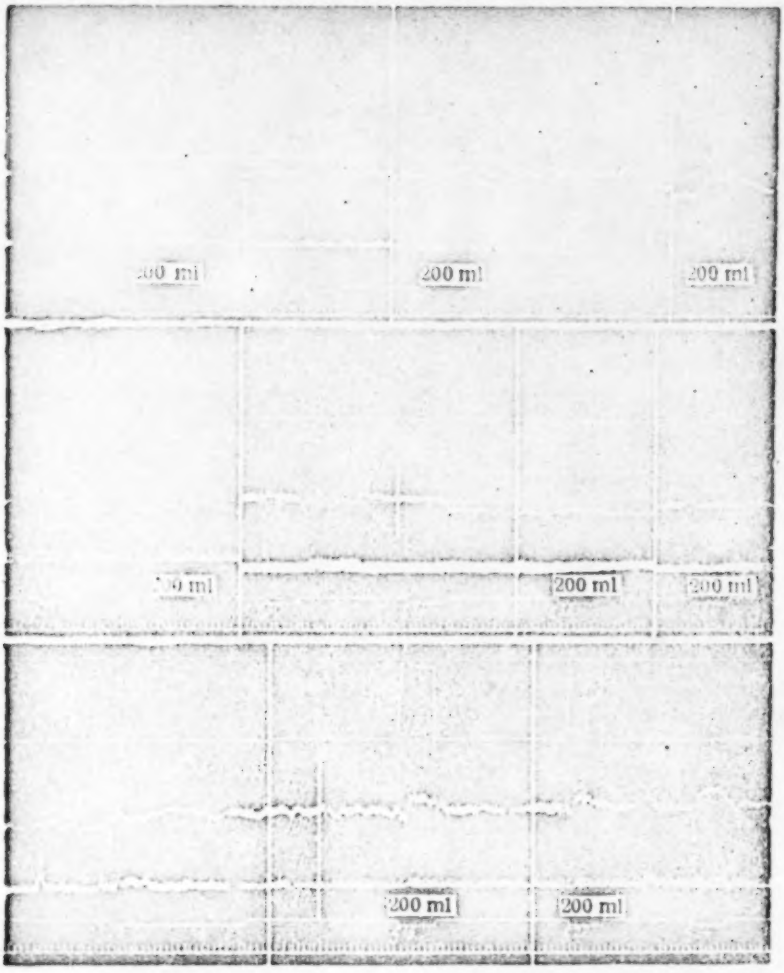

Fig. 3. Interoceptive reflexes under conditions of hypoglycemia provoked by insulin (2 units/kg). Druzhok experiment April 22, 1955. Reflexes from rectum – 2, 4, 5, 7, 11, 12, 13. Significance of tracings same as in Fig. 1. carotid artery clamped – ↓.

This normalizing influence of glucose permits a physiological explanation of its therapeutic actions in various phases of hypoglycemia. Basically, the glucose restores the normal relationships between the central nervous system and the internal organs which had been upset by the hypoglycemia.

In our opinion, our experimental data permits some discussion on the theoretical basis and practical application of insulin therapy in psychotic patients.

At present we know many psychic disturbances associated with pathological alterations in reception of impulses from the internal organs [1, 2, 4, 5, 13]. Judging from the clinical literature, the therapeutic insulin effect is obtained under conditions of suppression of the interoceptive reflexes. To this state of affairs testify such remarks as the need of lowering the glycemia quickly (intravenous insulin is advocated) with the goal of bypassing the period of preshock excitation and having the patient go into shock directly [12, 3]. Also, large doses of insulin are recommended with prolongation of the period of hypoglycemic shock [11], especially in cases of chronic schizophrenia [14], as well as simultaneous use of insulin and prolonged periods of interrupted sleep, the theory being to deepen the guarding reflex repression [6].

To all this must be added the fact that in hyperpyrexic therapy there is also suppression of the interoceptive reflexes as we have clearly demonstrated in our experiments on dogs having artificial fever [10].

Summarizing, it may be possible that the therapeutic basis for both fever and insulin therapy is the same. Basically, the temporary suppression of the interoceptive reflexes (especially if the interoceptive impulses are pathologic) permits a normalization of the functional interrelationships between the central nervous system and the internal organs and also a normalization of the functional state of the brain itself.

SUMMARY

Nonnarcotized dogs were employed in a study of hypoglycemic states. There was observed in these series experiments, first, an increased reflex response from both interoceptive stimuli from the rectum and stomach and also the baroceptive reflex from the carotid sinus. This was succeeded by a second phase of diminution and even total repression of the reflexes.

The dominating influence of the sugar blood level is pointed out. The reasons for some of the apparent differences in the results of the series and short experiments are discussed.

Finally, this temporary suppression of interoceptive reflexes is suggested as being the basis for the therapeutic effects of both insulin shock and fever therapy of schizophrenic patients.

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THE MECHANISM OF DYSENTERIC INTOXICATION

COMMUNICATION I INTEROCEPTIVE INTESTINAL REFLEXES IN THE PRESENCE OF EXPERIMENTAL DYSENTERIC INTOXICATION

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In the present study the chemoreceptors of the bowel were investigated as to the reflex effects produced on blood pressure and respiration under conditions of severe experimental poisoning with dysentery toxin.

The data in the literature [1, 2, 4, 7] concerning the effect various antigens produce upon the interoceptors seems inadequate to decide the question as to their participation in the pathogenesis of dysenteric intoxication.

EXPERIMENTAL METHODS

Fifty-two experiments were performed: 43 on cats being in a state of dysenteric intoxication and 9 on healthy animals.

The intoxication was produced by subcutaneous injection of Shiga dysenteric exotoxin (12-30 mouse LD per 1 kg weight). The chemoreceptors of the small intestine (45 experiments) and of the colon (7 experiments) were investigated with the use of the generally accepted technique. The experiments were conducted under urethane anesthesia given intravenously (4.1 cc of a 20% solution) some, 4-6 hours after injecting the exotoxin, some, 1,2, and 4 days later. The intestinal loop was extended from the general circulation and perfused with oxygenated Ringer-Locke solution at a temperature of 38-39°. The arterial pressure in the carotid artery and respirations were recorded.

The chemoreceptors were stimulated by a nicotine solution which was added to the perfusing fluid in a concentration of 1γ, 10γ, and 100γ per 1 cc of the solution. The toxin, sometimes 60-80 times the lethal dose, sometimes in individual instances 120 times the lethal dose, was placed upon the mucous membrane of the intestinal loop being perfused.

EXPERIMENTAL RESULTS

The first series of experiments were performed the 1st-2nd day after dysentery intoxication. Introducing the Shiga toxin into the lumen of the intestine had no effect on the arterial pressure, in 6 instances the pressure rose a minimal 2-3 mm mercury in the column and in only one instance was the rise equal to 10 mm mercury pressure.

After placing the poison upon the mucous membrane of the intestine, 7 out of 17 cases had increased reflex response to the nicotine, 6 – diminution or total suppression and in 4 – the reflexes were unaltered.

As an example of increased reflex response after placing the toxin, we can use the results obtained as represented in Fig. 1. As can be seen, introduction of the bouillon into the intestinal lumen ($\downarrow^1$) had almost no effect upon the arterial blood pressure (Fig. 1, a).

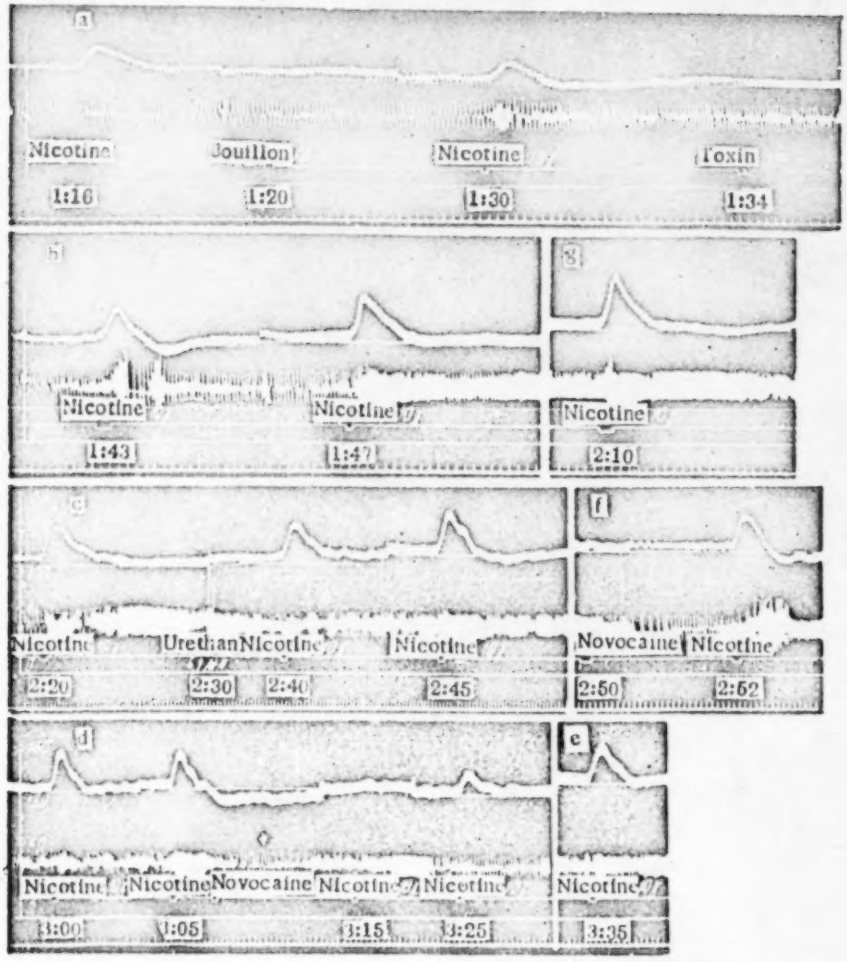

Fig. 1. Experiment of June 6, 1953. Cat, 3.2 kg. 2 days before the experiment 25 mouse LD of toxin per 1 kg weight were given subcutaneously (explanation in text).
Significance of the indicators (from above down): arterial pressure, base arterial pressure, respiration, marker of stimulator, zero line, time marker (5 seconds).

Nicotine did not alter the reflexes. After introducing the toxin into the lumen (1.0) of the intestine ($\downarrow^2$), the reflex responses were markedly reenforced (Fig. 1, b). Forty-six minutes after the introduction of the toxin, the same amount of nicotine (10γ) raised arterial pressure 54 mm of mercury, i.e., 38 mm more than before the toxin was introduced. After introducing the toxin, the amplitude of the respirations grew and the respiratory rate by 16-20 per minute, this being in contrast to the condition before the introduction of the toxin when 10γ of nicotine increased the respiratory rate only by 4-8 per minute. Deepening the narcosis somewhat decreased the reflex response to nicotine but they still conspicuously (by 14 mm mercury) exceeded the initial

responses. Introducing novocaine (2 cc 1% sol.) into the lumen of the cat's intestine did not alter much the reflex response of the arterial blood pressure. After the temporary abolition of chemoreceptor reflexes by injecting novocaine solution into the vessels of the isolated loop, the returning reflexes remained strong, considerably exceeding the initial response (by 18 mm mercury) even 2 hours after the toxin had been introduced into the intestinal lumen (Fig. 1,c).

Along with the increased reflex response to nicotine after the toxin had been placed on the mucous membrane of the poisoned cats, in 6 animals there was observed a depression or even a total abolition of reflexes. In 5 instances the reflexes were halved or even less as compared with base level; while in one the reflex response to nicotine was completely abolished.

In all 6 cases, within 15-60 minutes the reflex responses were fully restored. In the remaining 4 cases, there were noted no reflex alterations.

Analogous results were obtained in 7 experiments performed on the colon although the degree of reflex alterations was less. Apparently, this fact is associated with the lesser sensitivity of the colonic interoceptors to chemical stimulators [5, 9].

In this experimental series placing the toxin upon the mucosa within the first couple of days after dysenteric intoxication produced both a rise and a fall in the intensity of the reflex response to nicotine.

V. L. Troitsky and M. A. Tumanyan [8] report that, when dysentery toxin having a labeled phosphorus atom is given subcutaneously, the maximal urinary secretion of the toxin occurs after 4 hours. Using as a basis this statement, we conducted a series of studies on 10 animals 4-6 hours after poisoning them with the toxin.

In this series, 7 showed an increased reflex response to nicotine after the introduction of the toxin, while in 3 instances the reflexes remained unchanged. In no instance were the reflexes diminished. All these animals looked well; when sacrificed, there were no macrosocpic changes in the internal organs.

In the third series we studied interoceptive reflexes 4 days after poisoning the animals. As a rule, these animals became quite ill, frequently developed a diarrhea and lost muscular coordination. When sacrificed, there could be seen edema, hyperemia of both the serosa and mucosa of the bowel, hemorrhages, ulcerations and, sometimes, mucosal necroses. Analogous changes were seen in the stomach pylorus.

Of 9 experiments performed on these animals, in six the reflexes were diminished on the average some 4-6 mm of mercury with a restoration to normal after 7-35 minutes; in 2 instances the depressed reflexes failed in restoration, while in 3 – the reflexes remained unchanged.

Figure 2 presents the kymograms of one such experiment. Introducing the toxin into the intestinal lumen caused a slight rise in the arterial pressure and on this background the reflex response to nicotine (10γ) 20 seconds after introducing the toxin showed only a negligible alteration (Fig. 2, b). After 5 minutes there was a definite reflex depression (first, 6 and, then, 8 mm mercury; Fig. 2, b and 2, d). Thirty-five minutes after introducing the toxin (Fig. 2, g) the reflex response to nicotine failed to become restored; there was no increased reflex response in these experiments.

In 8 out of 9 control experiments conducted on well animals introduction of dysentery toxin into the intestinal lumen did not affect the arterial pressure or the respiration. In only one case introduction of the toxin led to a short, insignificant (3 mm mercury) rise of the arterial pressure. The reflex responses to nicotine remained either unaltered or changed but slightly. Of the 9 experiments in this series in six the reflexes stayed unchanged (Fig. 3), in 2 there was a slight reenforcement of the reflexes (response to 1γ of nicotine was increased 8 and 6 mm mercury) with a rapid restoration to normal (within 7-8 minutes). In one experiment the reflex response diminished by 4 mm mercury 13 minutes after introducing the toxin, by the 18th minute 1γ of nicotine had fully lost the capacity to elicit any response. In this experiment the reflexes remained lost.

In all the experiments, besides the arterial pressure, we recorded also the respiration but the small concentrations of nicotine used by us did not always increase respiration. For this reason we did not use the respiratory responses when analyzing the reflexes.

Thus we have established that the character of the reflex alterations depends on the phase of the intoxication.

The threshold dose for stimulating the chemoreceptors of the intestine in healthy animals, in our experiments, is 1 cc of a 10^{-6} (1γ) nicotine solution, this being in agreement with the greater material used by V. A. Lebedev [6].

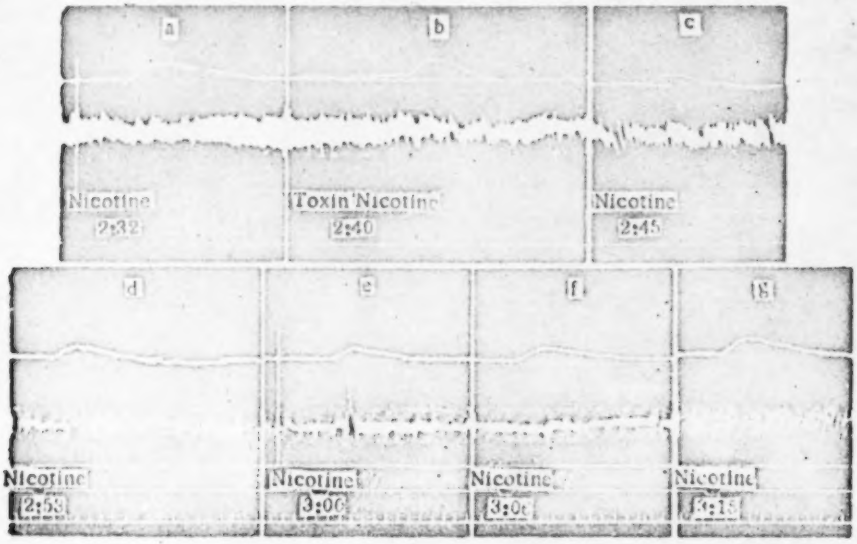

Fig. 2. Experiment of March 8, 1956. Cat weighing 2.9 kg. Toxin introduced 4 days previously (30 mouse LD per 1 kg). For clarification see text.
Significance of tracings (from above down): arterial pressure, initial level of arterial pressure, respiration, zero line, marker of stimulator, time interval (5 second marker).

Four to six hours after injecting subcutaneously the Shiga toxin, the threshold dose for nicotine stayed unaltered (1γ) remaining unchanged for 1-2 days. The intensity of the reflex response to 1γ of nicotine diminished over the first and second days for an average from 9.5 to 6 mm mercury. By the fourth day, a state of severe intoxication was apparent, the threshold rising to 10γ. There was no reflex response to 1γ.

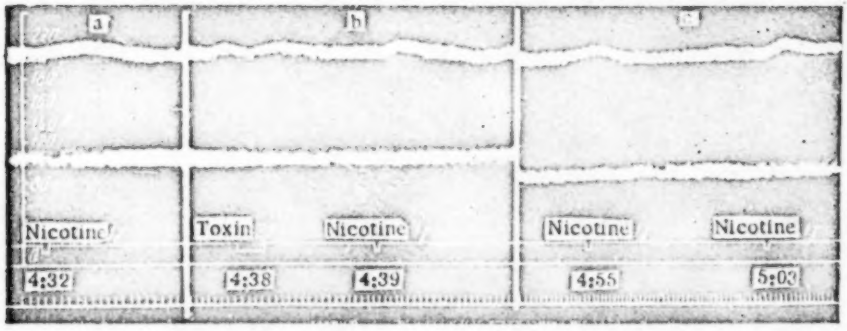

Fig. 3. Experiment of February 6, 1954. Cat weighing 4.150 kg, quite healthy. Explanation in text.
Significance of tracings as in Fig. 1. Time marker (2 seconds).

Our experiments suggest that even in the early phases of the intoxication offering the toxin to the intestinal receptors creates a greater sensitivity to the nonspecific chemical stimulator – nicotine.

When the dysenteric intoxication is fully developed, introduction of the toxin into the intestinal lumen provokes a temporary suppression of the reflex response to nicotine.

In healthy animals, analogous experiments produced no reflex alterations. Apparently, this indicates a greater sensitivity of the intestinal chemioreceptors to the dysentery toxin while the organism is suffering from dysenteric intoxication. A similar greater sensitivity of vascular receptors to antigens against a background of intoxication or sensitization to such agents was found by A. D. Ado and his associates [2].

There is also data indicating diminished sensitivity to acetylcholine after the dysentery toxin has acted upon the intestinal receptors [11].

The evidence presented seems to indicate that, during experimental dysenteric intoxication, the interoceptor chemical reflexes responding to chemical stimuli in the intestine become altered. It may be surmised that patients ill with dysentery have analogous changes which express themselves clinically [3].

Dysenteric toxins affect the interoceptive reflexes in a healthy organism very little if at all. For this reason, we agree with V. N. Chernova [10] who is of the opinion that irritation of the receptors in the intestine by dysentery toxins plays only a minor role in producing this disease.

SUMMARY

Reflexes obtained by perfusion of isolated intestinal loops with Shiga exotoxin and nicotine were used. Normal animals were tested first. Then, cats received large doses of Shiga dysenteric exotoxin subcutaneously and the isolated loop technique was followed within 4-6 hours in some animals; one, two and four days in others. Various concentrations of nicotine were used. Dysentery toxin was also placed on the mucosa of the loop as a definite maneuver.

The results were somewhat conflicting but there seemed to be an alteration of the reflexes in response to the stimuli. The time interval from the toxin injection is of great importance. Originally, there seems to be reenforcement of the reflexes; this being followed, later, by an inhibition.

The healthy animals were unaffected. These alterations are of such a nature that patients, already ill with dysentery, may have clinical manifestations analogous to those described here experimentally. It is doubtful if dysentery toxins play any role in the etiology of the disease.

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THIAMINE AND NICOTINIC ACID CONTENT OF VARIOUS SUBDIVISIONS OF THE BRAINS OF CHILDREN DYING OF CERTAIN INFECTIOUS DISEASES

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(Received September 6, 1955. Presented by Active Member Acad. Med. Sci. USSR Prof. N. A. Rozhansky)

Our preceding studies have shown that, in experimentally induced thiamine deficiency, various divisions of the nervous system of growing puppies had their thiamine content lowered down to 2-13 μg % (normal—140-180 μg %) [5].

In addition, these studies established the fact that in these thiamine deficient tissues the nicotinic acid level remains either within normal limits or is actually somewhat elevated.

The frequency and severity of the dystrophic alterations developing clinically in children dying eventually of dysentery led us to undertake an investigation into the amount of thiamine and nicotinic acid found in various subdivisions of the central nervous ystem of young patients succumbing to dysentery. We also did post mortem studies on cases in which the dysentery was accompanied by pneumonia; as well as cases in which the children had had pneumonia only.

In this study we used autopsy material taken from children dying between the ages of 0 to 2 years.

Material was taken from various divisions of the central nervous system as well as some other tissues of these children dying of dysentery (10 cases), dysentery complicated by pneumonia (12 cases), pneumonia (13 cases) and surgical ailments (5 cases).

The tissues of children dying suddenly of surgical complications were used as controls.

In all cases 6 areas of the brain were subjected to analysis: cerebral cortex (frontal lobe), caudate nucleus, optic colliculi, pons Varoli, medulla oblongata and also tissues from the liver, muscles, lungs and colon. These last were of interest as they represented the organs directly striken by the listed diseases. The total and free thiamine was determined by the thiochrome method, the total nicotinic acid — by the thiocyanobromide method.

The autopsy material was analyzed within 24-26 hours following the death of the child. Preliminary determinations had shown that when tissues were kept in a moist atmosphere at 24° for a whole day their thiamine or nicotinic acid content did not alter; longer storage of the material (48-72 hours) at these conditions decreased the amount of thiamine. The nicotinic acid remained unaffected.

Among the children dying of dysentery, 8 were in a hypotrophic state II. Of the 9 children dying of dysentery complicated by penumonia concerning whom we had a dietary history, only one was normotrophic, the others—hypotrophic. The children dying after surgery were well nourished; death was caused by peritonitis developing from bowel obstruction or appendicitis.

All the children had received antibiotics: sintomycin and penicillin. The amount of sintomycin received by each child had been small; only three children had been given 8 to 1 g for the total amount in the entire course of treatment, the other children receiving considerably smaller quantities. Also, the amount of penicillin given appeared to be relatively small. The sulfa preparations were utilized very rarely.

EXPERIMENTAL RESULTS

Thiamine content. As is well known from the literature, total thiamine in the brains of adults averages 140-160 μg %; in muscles and internal organs – 60 to 200 μg% [1, 6].

We were unable to find data on children.

Table 1 demonstrates the quantitative content of thiamine in the tissues of the dead children. The average content of thiamine in the brain divisions of children dying of dysentery was 3.7-6.9 μg%; in the colonic wall, muscle, lung and liver: 1.9, 3.3, 3.8, 6.2 μg% respectively.

TABLE 1

Thiamine Content of Brain Subdivisions and Various Organs

Name tissue	Thiamin content in μg% of tissues in children dying of			
	Dysentery	Dysentery compl. by pneumonia	Pneumonia	Surgical diseases
Spinal cord	4.5 (2—8)	5.4 (1—10)	8.3 (4—20)	12 (6—20)
Medulla oblon gata	6.5 (4—10)	5.5 (1—10)	10.0 (4—25)	12.7 (10—16)
Pons varoli	6.9 (3—12)	6.0 (1—15)	12.2 (5—40)	19.2 (8—25)
Ocular colliculi	5.8 (4—8)	4.5 (1—8)	9.5 (5—20)	15.2 (5—25)
Head caudate nucleus	4.8 (2—8)	5.7 (2—15)	9.0 (4—12)	31 (30—35)
Cerebral cortex	3.7 (2—5)	3.7 (0—8)	5.5 Traces —10)	33 (10—45)
Averages for nerve tissues	5.44	5.13	9.1	23.9
Liver	6.2 (2—10)	5.5 (4—8)	14.0 (3—60)	42.2 (25—60)
Lung	3.8 (1—7)	2.2 (0—4)	7.0 (0—15)	10.6 (4—15)
Colon	1.9 (0—5)	4.5 (0—25)	6.2 (0—12)	10.8 (0—30)
Muscle*	3.3 (0—6)	3.9 (2—8)	7.2 (2—20)	17.4 (8—35)

* The table presents the average values of total thiamine. In parentheses are given the limits of fluctuation of the thiamine concentrations.

When studying the peripheral tissues it was observed that the severest diminution of thiamine took place in the wall of the colon: in three out of ten cases thiamine had disappeared completely.

The thiamine content in the various subdivisions of the brain in children dying of dysentery complicated by pneumonia equaled 3.7-6 μg %. In the lungs of these children was found the smallest quantity of thiamine – 2.2 μg%; the fluctuation in the thiamine concentration in the colonic wall in 12 cases was 0-25 μg% (average 4.5 μg%), in muscle – 3.9 μg%, in liver – 5.5 μg%.

Similar quantities were found in the brain tissues of children dying of pneumonia (Table 1).

In all brain and other tissues of children dying of surgical causes, the thiamine content was much higher than in children dying of dysentery or pneumonia.

Nicotinic Acid content. In the brain tissues of children succumbing to dysentery the nicotinic acid content was 0.17-4.23 mg % (Table 2). The average content of this vitamin in the various brain subdivisions remained within normal limits (nicotinic acid in the brains of normal adults – 1.8-2 mg %). Nicotinic acid in the wall of the colon averaged 1.47 mg % in children dying of dysentery, the limits of fluctuation being 0.4-3.4 mg %. The accepted normal content of nicotinic acid in the colon is considered to be 1.3-2.4 mg%. In the muscles of the children of this group, nicotinic acid content averaged 1.69 mg % with fluctuations within the limits of 0.1-6.3 mg % (normal – 4.8-5 mg %). In this instance, there should be noted a diminution of nicotinic acid in the muscle tissues.

TABLE 2

Nicotinic Acid Content of Brain Subdivisions and Various Organs

Name of tissues	Nicotinic acid content in mg % in the tissues of children dying of			
	Dysentery	Dysentery complicated by pneum.	Pneumonia	Surgical diseases
Spinal cord	2.05[1] (0.33—3.33)	2.64 (1.73—5.53)	5.0 (1.15—8.91)	4.02 (1.5—7.6)
Medulla oblongata	1.86 (0.17—3.96)	2.61 (1.2—4.0)	4.93 (1.0—13.6)	4.5 (3.14—7.1)
Pons varoli	1.72 (1.0—2.33)	2.71 (1.3—4.22)	4.6 (1.5—9.3)	4.5 (1.7—8.24)
Ocular colliculi	1.96 (1.1—4.23)	2.44 (0.6—4.94)	5.8 (1.5—17.5)	6.15 (3.5—9.2)
Head caudate nucleus	1.87 (0.57—2.86)	2.64 (1.5—3.94)	4.5 (1.43—11.6)	5.31 (3.0—8.1)
Cerebral cortex	1.54 (0.5—2.03)	2.53 (1.63—4.77)	4.4 (1.95—9.0)	5.3 (4.1—7.0)
Average for nerve tissues	1.84	2.6	5.0	5.16
Liver	3.54 (1.2—6.13)	3.69 (2.13—6.2)	6.8 (1.5—18.6)	5.6 (2.04—10.0)
Lungs	2.06 (0.76—5.06)	2.8 (0.6—6.7)	4.1 (0.6—6.6)	3.2 (1.34—4.8)
Colon	1.47 (0.4—3.4)	2.6 (0.5—4.84)	4.9 (1.5—13.0)	2.76 (0.44—4.0)
Muscle	1.69 (0.1—6.3)	2.3 (1.13—5.2)	4.6 (1.58—12.74)	3.93 (2.8—5.4)

[1] The table presents the average values of total nicotinic acid. In parentheses are given the limits of fluctuation of the nicotinic acid concentrations.

In the lungs nicotinic acid averaged 2.06 mg % with fluctuations from 0.76 to 5.06 mg % which is practically normal (1.8 mg %).

The content of the vitamin in the liver averaged 3.54 mg % in the fluctuations being from 1.2 to 6.13 mg %, this being below the data in the literature (5.3-6.7 mg %).

In the brain tissues of children dead from dysentery complicated by penumonia, the nicotinic acid content fluctuated within the limits 0.6-5.53 mg %. The different brain subdivisions had sharply different amounts of this vitamin within them.

It was anticipated that the lung—the organ directly affected— would show alterations of the nicotinic acid content. Actually, the average was 2.8 mg % with fluctuations between 0.6 to 6.7 mg %— values somewhat higher than those obtained from children dying of dysentery only.

The nicotinic acid content of the colonic wall was somewhat greater than in the preceding group, averaging 2.6 mg % with fluctuations within the limits of 0.5-4.84 mg %. These figures can be evaluated as being within the upper limits of the normal seen in adults.

In muscles the nicotinic acid fluctuated between 1.13 and 5.2 mg %, the average being 2.3 mg %.

High nicotinic acid levels were discovered in the brain tissues of children dying of pneumonia or of acute surgical complications. In the first instance of the fluctuations were between 4.4 and 5.8 mg %, in the latter instance— 4.02-6.15 mg %.

Within the various subdivisions of the brain in children, dying at ages 0 to 2 years, from dysentery and dysentery complicated by pneumonia, there was discovered a marked lowering of thiamine— 3.7-6.7 mg %, while in those dying of pneumonia— 5.5-12.2 mg %.

In children of same age range dying of short term surgical conditions the thiamine content proved to be high equaling 12-33 mg %.

The nicotinic acid content of the brain and other organs in children dying from dysentery was lowered only minimally while in other conditions it was within normal limits.

This severe lowering of thiamine content in children dying of dysentery agrees with the experimental data obtained from puppies put into a prelethal state by prolonged thiamine deprivation. This gives substantial basis for the belief that thiamine insufficiency plays an important role in the pathogenesis of dysentery.

SUMMARY

The vitamins thiamine and nicotinic acid were studied in the tissues of children dying from dysentery alone, dysentery complicated by pneumonia and various acute surgical conditions. Specific subdivisions of the brain as well as some internal organs were examined. The age range was 0 to 2 years.

Nicotinic acid was not found to be diminished seriously, if at all. Thiamine underwent marked depletion, particularly in the central nervous system. This is in agreement with experimental data obtained on studies performed with growing puppies.

There is substantial reason for the belief that thiamine depletion plays a role in the pathogenesis of dysentery.

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BIOCHEMISTRY AND BIOPHYSICS

SITE OF CARNOSINE BIOSYNTHESIS IN THE ORGANISM

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As a result of studies conducted in recent years under the direction of S. E. Severin, considerable progress has been made in clarifying the physiological role of carnosine and anserine. The data obtained indicate participation of these substances in the aerobic and anaerobic processes of carbohydrate conversion in muscle tissue, leading to formation of high-energy compounds [2, 6, 7]. Until now, however, the question of pathways and site of synthesis in the organism of these dipeptides has remained unclear.

In 1950-1952 N. A. Yudaev obtained the first experimental data on formation of carnosine and anserine in the organism by way of condensation of the amino acids forming the dipeptides [8, 9], the conclusion being reached that carnosine synthesis occurs in the muscle tissue itself [8].

In later studies of other authors the chief part in this synthesis was, however, referred to the liver [1, 4, 21].

In the present paper the question of the site of carnosine synthesis and the role of the liver in this process was studied. The method of isotope indicators was used in combination with operations of functional exclusion of the liver and complete hepatectomy. In the course of the work labeled histidine-$2C^{14}$ was synthesized also.

METHODS

Labeled histidine-$2C^{14}$ was synthesized according to Borsook's modification [13], the basis of which is the method of Ashley and Harrington, developed for obtaining thiohistidine [12]. Certain steps were carried out by directions proposed by Heath, Lawson and Rimington, in which yields of a number of intermediate products were markedly improved [15].

Young rabbits of 3-8 days and adult rats were used for experiments. The labeled amino acid was administered subcutaneously in the amount 2000-5000 impulses per minute per 1 g body weight. Muscles of the rear extremities were used for study, and also spinal muscles in the young rabbits. Muscle tissue was freed of fat and connective tissue, ground with 4 volumes of 5% trichloroacetic acid and centrifuged. Carnosine was isolated from the centrifugate and purified of amino acids. This was necessary since according to literature data the second carbon atom of the imidazole ring of histidine can be utilized for building the carbon skeleton of certain amino acids [20]. Radioactivity of proteins in the precipitate was investigated.

Carnosine was purified by twice precipitating the dipeptide with Hopkins' reagent (10% solution of $HgSO_4$ in 5% H_2SO_4). The mercury precipitate was washed 3-4 times with water to which a small amount of the precipitating reagent (1 : 50) was added and was decomposed with hydrogen sulfide. The acid solution, containing SO_4 ions, was neutralized with cold saturated $Ba(OH)_2$ solution and the precipitates of barium sulfate and mercury sulfide filtered off. The filtrate obtained was evaporated to a small volume and chromatographed twice. Chromatography was conducted according to the method previously described [10]. Usually an extract of two samples was applied to a sheet of Wattman No. 2 paper 56×30 cm on the edge of the broad side of the paper.

As the first solvent 80% aqueous phenol was used, in which carnosine was separated from the main mass of remaining amino acids. The second solvent was 80% aqueous phenol saturated with HCl vapors [11]. For the second chromatographing carnosine was extracted from the first chromatogram 7-10 times with hot water, the extract was filtered, evaporated to a small volume and applied to a new sheet of paper. Previous to extraction of carnosine from the chromatogram, solvent was removed. The phenol was first removed at room temperature in a stream of air, and chromatograms were treated first with ether 2-3 times in a bath, then 7-10 times with acetone in a Soxhlet apparatus. In extracts obtained after the second chromatographing (purity of the isolated carnosine was 90-95%) the radioactivity and amount of carnosine was determined. Carnosine was determined by the diazo reaction as modified by Meshkova [3]. Protein was treated according to the previously described method [17]. Then 10-mg batches of it were prepared and placed on foil targets. Radioactivity was determined with a Geiger-Muller counter.

EXPERIMENTAL RESULTS

The data obtained on incorporation of histidine-$2C^{14}$ into muscle dipeptides of normal animals are given in Table 1.

As seen from this table, 24 hours after single injection of histidine-$2C^{14}$ in the amount 2000-5000 impulses per minute per 1 g body weight the radioactivity of muscle carnosine of young rabbits was 700-800 impulses per minute per 1 mg dipeptide. Repeated injections at 24-hour intervals led to increase in dipeptide activity; however, the extent of utilization of histidine-$2C^{14}$ scarcely changed, which is indicated by the constancy of the

TABLE 1

Incorporation of Histidine-$2C^{14}$ into Carnosine and Proteins of Muscles of Young Rabbits and Adult Rats

Exp. No.	Exp. animals	Animal wt. in g)	No. of injections	Time after last injection (in hrs.)	Carnosine activity: In imps. / min. per 10 mg	Carnosine activity: in ratio of activity 1 mg carnosine to activity histidine admn. per 1 g body wt.	Protein activity (in imps./ min. per 10 mg)
1	Young rabbits	60	1	24	601	0.261	—
2	Same	72	1	24	824	0.356	65
3	"	149	1	24	739	0.246	57
4	"	75	2	24	972	0.226	—
5	"	185	2	24	1285	0.240	90
6	"	113	3	24	1541	0.270	92
7	"	190	3	24	1532	0.170	117
						Average 0.253	
8	Rats	270	1	4,5	842	0.168	—
9		252	1	24	726	0.242	10.6

ratio of activity of 1 mg carnosine to activity of the histidine administered per 1 g body weight. This fact indicates slow loss of label from the carnosine, since theoretically with increase in number of injections this ratio should decrease. Labeled histidine was also incorporated into muscle carnosine of adult rats.

As a result of hydrolyzing the carnosine it was found that the dipeptide activity was due to the histidine residue.

Preliminary calculations showed that muscles of young rabbits retain 5-7% of the amount of histidine-$2C^{14}$ administered, 50-60% being in the nonprotein fraction chiefly carnosine.

Unlike carnosine we could not detect the appearance of radioactivity in muscle anserine of adult rats after injection of histidine-$2C^{14}$ even upon administration to animals of 30 times greater activity (100,000 imp./min per 1 g body weight).

The cause of the negative results is apparently the slowness of the processes of methylation of histidine. Possibly the methylation is accompanied by formation of an intermediate product, which proceeds at a slow rate, thus limiting the rate of the process as a whole. It was recently shown that after administration to rats of labeled C^{14} in the methyl group of methionine the label appears in anserine in 4 hours [18]. Comparison of the data obtained with ours warrants the assumption that in this case either a precursor of methylhistidine present in tissues could be methylated, or carnosine.

In recent years the incorporation has been shown of labeled β-alanine-$1C^{14}$ and histidine-$2C^{14}$ into carnosine and anserine of chicks and young rats and the incorporation of labeled 1-methylhistidine-C^{14} into anserine of the same animals [14, 19]. The data obtained together with the incorporation of histidine-$2C^{14}$ into carnosine of young rabbits and adult rats which we observed, support the view expressed above of synthesis of the dipeptides in the organism by way of condensation of their constituent amino acids.

In order to clarify the question of the site of carnosine formation and the role of liver in this process, rats were subjected to the operation of functional exclusion of the liver. Animals were used for experiment in which after ligating appropriate vessels the gastrointestinal tract was removed entirely, and vessels were also ligated which supply the liver [5]. Animals served as controls in which the abdominal cavity was opened simultaneously with the experimental and subsequently closed.

After injection of labeled histidine in the amount 5000 impulses per 1 g body weight, it was found that intensity of incorporation of this amino acid into carnosine in animals with liver excluded from the circulation and deprived of gastrointestinal tract was 1½ times greater than in sham-operated animals. Study of radioactivity of liver protein showed that these values in experimental animals were less than 5% of the respective values of control animals. At the same time radiohistidine incorporation into muscle proteins of experimental animals was even a little increased. Thus, although the liver was not completely removed from the animal, the administered histidine scarcely entered it (Table 2). The observed increase in labeled histidine incorporation into muscle carnosine of experimental animals is apparently explained by the sharp decrease in cleavage of the administered amino acid, as well as by the fact that it is not utilized for synthesis of proteins of the removed organs.

TABLE 2

Histidine-$2C^{14}$ Incorporation into Carnosine and Proteins of Muscles and Liver of Sham-Operated Animals and Animals with Functional Exclusion of Liver

Exp. No.	Time after histidine administrat. (in hrs.)	Sham operation				Liver exclusion			
		Animal wt. (in g)	activity			Animal wt. (in g)	activity		
			carnosine (in imps. / min. per 10 mg)	muscle protein (in imps. / min. per 10 mg)	liver protein (in imps. / min. per 10 mg)		carnosine (in imps. / min. per 1 mg)	muscle protein (in imps. / min. per 10 mg)	liver protein (in imps. / min. per 10 mg)
1	10	—	—	—	—	313	3161	32.8	7.25
	14	275	—	16.1	404.4	311	2719	21.3	15.9
	19	270	995	16.5	416	313	2742	29.5	12.5
	21	263	1646	9.9	400	303	3223	36.5	14.4
2	13	—	—	—	—	256	1014	15.9	8.0
	13	—	—	—	—	273	1585	19.5	3.5
	15	215	975	16.5	201	—	—	—	—
	15	220	686	15.4	220	—	—	—	—
	20	251	983	17.9	236	237	1863	25.5	3.8
	21	208	1021	13	226	242	1524	26.0	7.0

In the next series of experiments the operation was performed in two steps with an interval of 2-3 weeks, which permitted complete removal of not only the gastrointestinal tract but also the liver [16]. In the hepatectomized animals the kidneys were also removed with the purpose of determining their role in carnosine synthesis. Study of incorporation of radioactive histidine into carnosine in animals thus operated gave results analogous to those obtained in the preceding series of experiments (Table 3). The results of our experiments on animals

subjected to the operation of functional exclusion of the liver coincide very well with recent data on the fact that labeled β-alanine is incorporated at a greater rate into muscle carnosine and anserine of rats subjected to operative removal of $^2/_3$ of the liver. The authors of these studies concluded that they did not succeed in obtaining information on whether formation of the dipeptides proceeds in the liver; they also point out that in order to establish the site of their synthesis, additional studies are necessary [14].

TABLE 3

Carnosine Biosynthesis in Hepatectomized Rats

Exp. No.	Hrs. after admin. of labeled histidine	Sham operation			Hepatectomy		
		animal wt. (in g)	carnosine activity		Animal wt. (in g)	carnosine activity	
			in imps./min. per 1 mg.	in ratio of activity 1 mg carnosine to activity histidine admin. per 1 g body wt.		in imps./min. per 1 mg	in ratio of activity 1 mg carnosine to activity histidine admin. per 1 g body wt.
1	3.5	—	—	—	284	617	0.124
	4.0	—	—	—	255	971	0.204
	7.0	—	—	—	405	1208	0.240
2	2	—	—	—	255	201	0.042
	3.5	250	584	0.117	—	—	—
	4.0	280	984	0.200	280	550	0.112
	5.0	280	1016	0.207	262	1238	0.248
	6.5	292	894	0.177	307	1764	0.353

The data obtained on the increased rate of histidine-$2C^{14}$ incorporation into muscle carnosine of rats with functional exclusion of the liver and on renewal of this dipeptide in hepatectomized and nephrectomized animals permit the conclusion that liver, kidneys and gastrointestinal tract do not participate in carnosine synthesis and speak in favor of its formation in muscle tissue.

I am very grateful to V. O. Maimind for guidance in synthesis of histidine-$2C^{14}$ and for supplying a preparation of radioactive potassium thiocyanate.

SUMMARY

Radioactive histidine was used to investigate the place of synthesis of muscular carnosine. In rats with the liver functionally excluded synthesis of muscular carnosine by the inclusion of the radioactive amino acid progresses rapidly. Renovation of the dipeptide took place on both hepatectomized and nephrectomized animals. From the data gained the conclusion can be made that liver, kidneys and gastrointestinal tract do not participate in the synthesis of carnosine, its formation taking place in the muscular tissue.

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PHARMACOLOGY

THE EFFECT OF CORAZOLE* ON THE PERIODIC ACITIVITY OF A "HUNGRY" STOMACH

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Extra-digestive gastric contractions were first observed by A. M. Cheshkov [7], in experiments on dogs. V. N. Boldyrev [1] studied this activity of the gastrointestinal tract systematically and in detail. V. N. Boldryev and other authors have shown that the increased motor activity in the body of a hungry animal or man is connected with a series of physiological reactions. The regular appearance and accurate alternation of the active and resting periods of all the periodic activity components indicates that these periodic changes in the body are regulated by one central mechanism.

The existence of central influences on the stomach has been proved by direct experiments with mechanical denervation of the stomach. It was established that the periodic gastric activity ceases when the vagus nerves are cut. As the vagus nerves regenerate, the "hungry" movements of the stomach are restored [6].

Therefore, according to contemporary opinion, the periodic activity of the stomach is regulated by the central nervous system. The regularity and inertia in the appearance of this function suggest that it is the lower sections of the central nervous system which directly regulate the periodic activity of the stomach. The cerebral cortex can adjust and modify the function in question according to environmental conditions and to the condition of the body [2, 3, 5].

Studying the periodic contractions of the stomach can be quite helpful in ascertaining the mechanism and typical effect of therapeutic substances on the lower sections of the central nervous system.

In this work, we studied the effect of corazole, which has a stimulating effect on the central nervous system, on the periodic activity of a "hungry" stomach.

EXPERIMENTAL METHODS

The experiments were done on two dogs, Secret and Tarzan, which had Basov's gastric fistula. The animals were experimented upon 18-20 hours after their last meal. Any food that still remained in the stomach was washed out with a lukewarm physiological solution. The contractions were recorded with a rubber balloon, which was connected to Marey's capsule by a water-air transmission with a system of rubber tubes. The gastric contractions were registered on a kymograph-smoked tape, with a slow cylinder rotation (1 turn per 6-8 hours). This slow rotation was accomplished with the help of a small, synchronized Warren's motor, which was joined with the rotating mechanism of the kymograph by a system of cog-wheels. The low speed of the kymograph cylinder's rotation made it possible to record an entire experiment or most of it on one tape (duration of experiment: 6-10 hours).

* Russian trade name.

Fourteen control experiments were done and thirty-five with the corazole injection.

In the dog Secret, the active and resting periods occurred with a strict rhythmicity in the control series of experiments. Four to five active periods occurred, lasting from 16-25 minutes during 7-8 hours of observation. The resting periods lasted from 70-95 minutes.

Slightly more variation in the duration of the active periods and the intervening pauses was observed in the control experiments with Tarzan. The contraction periods lasted 14-30 minutes, and the resting periods, 65-100 minutes.

In view of the fluctuating indices of periodic gastric activity in the control experiments, we conducted preliminary observations for a period of 3-4 hours before each experiment, as well as those done in the control experiments, and only administered the preparation to be tested after we knew the character of the contractions on the day of the experiment. Corazole was injected subcutaneously during the pause between the contractions, 15-25 minutes after the end of the last active period.

EXPERIMENTAL RESULTS

We first examined the effect of corazole in a dose of 20 mg/kg, which, according to data in the literature, has a marked stimulatory effect on the central nervous system. The injection of this dose of corazole caused such a considerable protraction of the resting period that, in some experiments, the alternating action period disappeared completely. In some experiments, the contraction periods became much shorter, and the amplitude of the contractions decreased. Often the action period succeeding the protracted pause also was lengthened (see Table and Fig. 1).

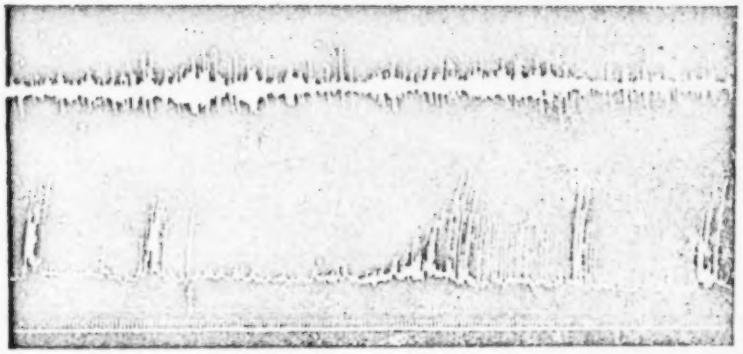

Fig. 1. Effect of 20 mg/kg of corazole on periodic gastric activity in Tarzan. Curves from top to bottom show: respiration, periodic gastric contractions; indication of time (in 4 minute marks). The arrow shows the corazole injection.

Therefore, when testing the effect of 20 mg/kg of corazole on the "hungry" movements of the stomach, we discovered that this dose first suppressed motoricity up to complete inhibition of the periodic contractions for 2-4 hours, but then increased gastric motor activity.

There are indications in the literature that corazole can paralyze the postural reflexes, the labyrinth reflexes and the righting reflex [8, 9]. However, since this type of effect was only observed when very large, convulsive doses of the preparation were administered, the inhibition observed may have been due to the paralysis of the nerve centers.

An inhibitory effect on models of periodic activity was obtained from a comparatively small dose of corazole, which did not cause convulsions or any change in the outward behavior of the animals. In this case, it is difficult to believe that gastric motor activity was inhibited because of nerve center paralysis. It is more probable that, in our experiments, pessimal inhibition was caused by the addition of corazole's stimulatory effect to the already stimulated centers regulating the "hungry" motoricity of the stomach. If our proposition is correct,

then smaller doses of corazole should also have a stimulating effect on this function. There was no noticeable effect on the character of the periodic gastric contractions in either of the dogs caused by a dose of 5 mg/kg of corazole. When 10 mg/kg was injected, changes were observed in the dog Tarzan, but they were unstable in character: in three out of five experiments, the pause either became shorter or the action periods longer.

TABLE

Duration of Resting and Action Periods in Minutes in the Dog Tarzan with 20 mg/kg of Corazole Injected

No. experiments	Duration of resting period		Duration of action period	
	Before corazole injection	After corazole injection	Before corazole injection	After corazole injection
1	102	228	18	80
2	108	156	19	43
3	94	116	21	19
4	88	216	16	132
5	88	220	16	50
6	86	260	25	40

The stimulating effect of corazole on the "hungry" movements of the stomach was clearly manifested in the dog Tarzan when a dose of 15 mg/kg was used: the contraction periods became 5-38 minutes longer, an average of 21 minutes for five experiments (Fig. 2).

Fig. 2. Effect of 15 mg/kg of corazole on periodic gastric activity in Tarzan. The curves are the same as in Figure 1.

In Secret, unlike Tarzan, this dose of corazole did not have this type of effect in any of the experiments. In two out of four experiments, this analeptic had an immediate inhibitory effect of the gastric motoricity, which was indicated by a certain decrease in the duration of the action period (7-9 minutes).

Therefore, the experiments with the injection of corazole in small doses showed that this analeptic can have a stimulating effect on the periodic activity of a "hungry" stomach, within a definite range of doses, which was shown most clearly in the experiments on Tarzan.

Therefore, the use of periodic gastric activity as an index of the typical action of corazole shows the diphasic character of its effect on the central nervous system. It is particularly certain that small doses of corazole (15-20 mg/kg), which do not have a convulsive effect, can have both a stimulating and an inhibitory effect on the central nervous system. It is possible that the inhibitory effect depends on the development of pessimal

inhibition in the centers regulating the periodic activity of the "hungry" stomach. It is interesting that, in conformity with our data, the inhibitory effect of corazole could also be observed on a model of calomel hypersecretion. The centers of the brain stem which regulate intestinal secretion appeared in a condition of maximal stimulation when an intestinal loop, isolated according to Thiry-Della, was stimulated with calomel [4]. In both cases, the existing condition of preliminary stimulation evidently aided the development of the inhibitory phase of corazole's effect on the central nervous system.

SUMMARY

It has been experimentally shown that the effect of corazole on the "hungry" movements of the stomach depends on the dose given. Large but not convulsive doses (20 mg/kg) cause an inhibitory effect resulting, probably, in pessimal inhibition developing in the central nervous system. Smaller doses (10-15 mg/kg) exert a stimulating effect leading to prolonged peristalsis.

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THE SELECTIVE EFFECT OF CERTAIN CHEMICAL STIMULTANTS ON THE INTEROCEPTIVE REFLEXES

Article IV. THE EFFECT OF STREPTOMYCIN ON CHEMORECEPTOR AND MECHANORECEPTOR REFLEXES IN CONDITIONS OF DECEREBRATION

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Data from experimental research has shown [1, 2] that the general effect of streptomycin is to act electively on definite mechanisms in the central nervous system. Its action, which is, as a rule, inhibitory, affects primarily that part of the nervous system into which the impulses from the chemoreceptors enter.

We conducted a series of experiments similar to that described in Article II [2] on decerebrated, healthy animals in order to definitely locate the part of the central nervous system affected by the action of streptomycin.

EXPERIMENTAL METHODS

The experiments were conducted on male cats. The animals were anesthetized with ether and fastened to a table, then both carotid arteries and the trachea were dissected free. The left carotid artery was ligated and used to measure blood pressure. A clamp was applied to the right carotid artery in order to prevent hemorrhage when the brain was transected. Then the animals were fastened dorsal side up on the table; a curved incision was made into the skin of the left temporal region of the head, and the temporal bone was exposed; a hole 2 cm in diameter was drilled into it, an incision was made into the dura mater and the brain was transected between the superior and inferior colliculi of the corpora quadrigemina.

In almost all the animals, the operation was attended by weakened respiration and more or less developed muscular rigidity. We did not begin the experiments until 60-80 minutes after the operation. Usually, an even background of blood pressure and respiration had been established by this time.

Blood pressure and respiratory reflex reactions from the chemoreceptors of the small intestine were caused by stimulating the latter, isolated from circulation (according to V. N. Chernigovsky), with acetylcholine (10^{-4}), nicotine (10^{-5}) and carbon dioxide (10^{-5}) under perfusion conditions.

The walls of the bladder were distended with air (70-80 mm of mercury pressure) to examine the reflex reactions from the mechanoreceptors.

The blood pressure in the left carotid artery was recorded and respiration was recorded by means of a Marey's capsule.

The blood pressure and respiratory reflexes caused by stimulation of the chemoreceptors and mechanoreceptors were studied in 5 experiments. The same reflexes from the intestinal chemoreceptors alone were studied in 4 experiments.

Streptomycin was injected intramuscularly in a dose of 200,000 units. The reflexes were examined before the preparatory injection and then every 15-20 minutes after the injection for an average of $1^1/_2$ hours.

EXPERIMENTAL RESULTS

Twenty minutes after the streptomycin injection, the blood pressure fell sharply in two experiments, becoming, by the end of the experiment (after 2 hours) 35.5 and 27.4 % of the original level. In 4 experiments, the blood pressure did not fall as sharply after the streptomycin injection, but fell an average of 18.6%. In one experiment, the pressure remained practically the same, while, in two experiments, the blood pressure curve rose 9.9 and 12.7%.

The original reflex reactions from the experimental zones were considerably greater than in the experiments using anesthesia (this has already been pointed out by V. N. Chernigovsky (1941), V. A. Lebedeva (1947), G. I. Kovaleva (1949) and other authors). How these reactions changed under the influence of streptomycin is described as follows.

The vasomotor reflex to acetylcholine stimulation of the chemoreceptors was studied in all 9 experiments. After the streptomycin injection, it decreased more than 50% in 4 experiments (Fig. 1), in two other experiments,

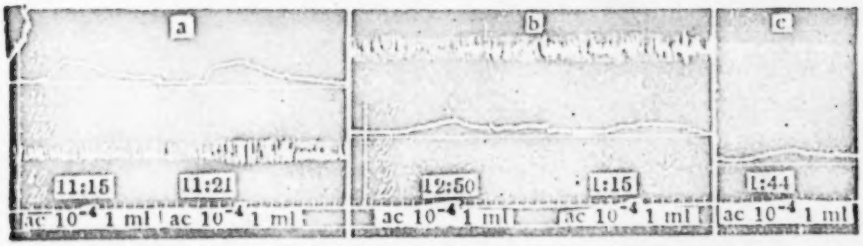

Fig. 1. Effect of streptomycin injected intramuscularly on blood pressure level and on reflexes from chemoreceptors of the small intestine.
a) before injection; b,c) after streptomycin injection (200,000 units). Curves from top to bottom indicate: a) blood pressure, respiration, indication of time in 5 second marks, indication of stimulation; b, c) respiration, blood pressure, indication of time, indication of stimulation.

by 23 and 37.5%, and it disappeared completely in two experiments. In one experiment, the vascular reflex to acetylcholine was reversed 20 minutes after the antibiotic injection (depressor reaction), but, by the end of the experiment, had regained its usual form (pressor effect) and even slightly increased.

The blood pressure reflexes caused by the introduction of nicotine into the perfusate (8 experiments) were very large before the streptomycin injection, but became considerably smaller after the injection. In 5 experiments, the reflex decreased more than 50% with the introduction of nicotine into the perfusate. In one experiment, an hour after the streptomycin injection, the reflex disappeared completely upon the introduction of nicotine into the perfusate (Fig. 2); in one experiment, it decreased by 30.8%. This reflex also increased spontaneously before the streptomycin injection. Finally, in one experiment, the reflex to nicotine increased 38% after the streptomycin injection.

Reflexes to carbon dioxide were studied in 5 experiments. In two of them, the blood pressure reflex was completely suppressed after the streptomycin injection; in one experiment, the reflex decreased more than 50%, in another, 30%, and in the last experiment remained almost the same.

The reflexes from the bladder mechanoreceptors were also studied in 5 experiments. The results of these experiments confirmed the rule which we discovered in previous studies. In 3 experiments, the vascular reflexes occurring when the bladder mechanoreceptors were stimulated remained the same after the streptomycin injection, and they became only slightly smaller in two experiments (Fig. 3). As a rule, in these experiments, the reflexes from the small intestine chemoreceptors either completely disappeared or were greatly inhibited.

Streptomycin caused the respiratory reflexes in the decerebrated animals to change in the following manner. The reflex to acetylcholine was inhibited in 3 experiments and unchanged in 5, i.e., the intensity of increase in amplitude and frequency of the respiratory movements was the same before and after the injection of the preparation. When the chemoreceptors were stimulated with nicotine, the respiratory reflex was inhibited in 5 cases and remained unchanged in 3.

The reflexes to carbon dioxide were tested in 5 experiments. In two experiments, after the streptomycin injection, the frequency of the respiratory movements became lower and the respiration amplitude remained the same. In one experiment, the respiratory reflex disappeared completely, and in two experiments, streptomycin caused no change in the respiratory reflex.

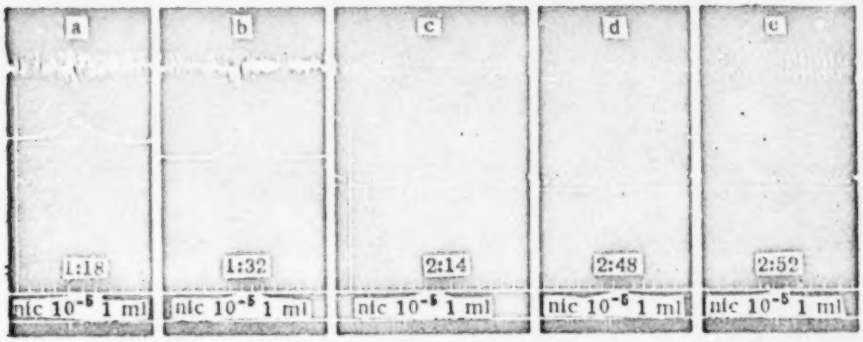

Fig. 2. Effect of streptomycin injected intramuscularly on reflexes from chemoreceptors of the small intestine.
a, b) original reflexes; c, d, e) after streptomycin injection. Curves (from top to bottom) are the same as in Fig. 1.

Out of 5 experiments with bladder mechanoreceptor stimulation, the respiratory reflex was only inhibited in one, while in the other experiments, the reflex did not change.

Therefore in experiments on decerebrated animals, streptomycin has the same effect on the reflexes studied as in experiments using narcosis. In this case also, in a large majority of experiments, streptomycin caused the absolute magnitude of the blood pressure reflex to decrease, the respiratory reflexes occurring upon stimulation of the small intestine chemoreceptors to be inhibited, and no change or only slight inhibition of the same reflexes occurring in response to stimulation of the bladder mechanoreceptors.

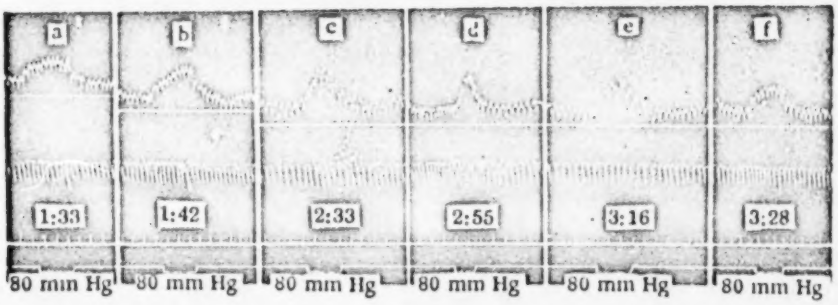

Fig. 3. Lack of change in reflexes from the bladder mechanoreceptors in connection with the streptomycin injection.
a, b) original reflexes; c, d, e, f) after streptomycin injection. Curves (from top to bottom) are the same as in Fig. 1.

The data obtained indicate that the selectively inhibitory effect of streptomycin on the afferent course from the chemoreceptor fields is realized in the central nervous system at a level located below the midbrain. Special electrophysiological studies are necessary to more exactly determine the area directly influenced by the antibiotic's action.

SUMMARY

Experiments on cats have shown that the selective inhibitory effect of streptomycin on the chemoreceptive afferent paths is accomplished at the level situated below the mesencephalon.

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MICROBIOLOGY AND IMMUNITY

ON THE QUESTION OF THE RAPIDITY OF DEVELOPMENT AND DEGREE OF IMMUNITY AGAINST TETANUS AND GAS GANGRENE WITH DIFFERENT METHODS OF IMMUNIZATION

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One of the important problems of contemporary practical immunology appears to be the investigation of methods of active immunization which would make it possible to produce a sufficiently high-grade immunity as rapidly as possible after the introduction of the antigen.

Among the recent studies dedicated to the study of the problem referred to, it is necessary to mention the investigations of Lemetayer, Raynaud and others [11], showing that by the injection of large doses of toxoid it is possible to build up specific resistance in animals, not previously exposed to immunization, almost immediately after the injection of the toxoid. The resulting insusceptibility is to be explained, not by the formation of antibodies, but rather to a blocking action of the toxoid itself. Similar observations were made by Krech [10].

In 1935, Ramon, Lemetayer and Borella [16] showed that the daily injection of fractional doses of toxoid 5-7 times a day could call forth antitoxin in the blood of guinea pigs on the 11th-15th day; it proved most effective to inject the toxoid always in the same place.

In the investigations previously carried out in our laboratory [1] it was shown that by fractional injection of tetanus toxoid at intervals during the course of a day it is possible to build up a definite resistance to tetanus toxin by the 12th day after immunization. For this the optimal schedule appeared to be 9 injections at 3-hour intervals all in the same place.

On the other hand, however, Kovtunovich [7], making use of the other immunizing programs, in experiments on mice could not demonstrate the development of early resistance to tetanus toxin.

Recently immunization with adsorbed toxoid has enjoyed widespread use. In comparison with present methods this procedure possesses a series of advantages.

However, a number of questions concerning the peculiarities of the increase in grade of immunity have even yet been little investigated. In particular, the question of the possibility of increase in degree of immunity as soon as possible by use of adsorbed toxoid has hardly been studied.

All accounts prompted us to compare the effectiveness of initial immunization, keeping in mind the grade of immunity and the time of its development, for various schedules and methods of employment of toxoid. During this work we studied immunization, not only against tetanus, which presented itself as a model in the above-mentioned investigations, but also against gas gangrene.

The work was carried out on white rats. There were studied fluid tetanus toxoid and toxoid absorbed on aluminum hydroxide, purified tetanus toxoid, and Cl. perfringens toxoid. To test the resistance of the animals we employed tetanus toxin and Cl. perfringens toxin. All preparations were obtained from the Anaerobic Division of IEM AMN USSR* (Institute for Experimental Medicine of the USSR Academy of Medical Sciences).

In the first series of experiments crude tetanus toxin, series No. 336, was used. It contained about 60-70 combining units (CU) in 1 ml. We injected the toxoid once into the left hind leg of the animals of the first group; into the animals of the second group we injected the toxoid fractionally (9 times), always in the same place, at 3-hour intervals, in the course of the day (indicated as optimum by the schedule of "most rapid immunization" already described; into the animals of the third group we made a single injection of the same dose of toxoid, adsorbed on aluminum hydroxide (5 mg Al_2O_3 to 1 ml toxin; the toxoid was completely adsorbed). To elucidate the question of the significance of different modes of administration of the toxoid, in all three groups we injected some animals subcutaneously and some intramuscularly. We estimated the effectiveness of the immunization by the resistance of the animals, which we challenged on the 12th day by the injection of 2.5 MLD of toxin into the muscles of the extremity opposite the one used previously (right foot).

TABLE 1

Resistance of Rats to Tetanus Toxin after Immunization with Crude Tetanus Toxoid Series No. 336 Following Various Methods of Employing Toxoid

No. of Group	Program of Immunization	Mode of Employing Toxoid	No. of Animals in Experiment	Toxic Reaction	Died	Mean Survival Time of Animals which Succumbed (in days)
1	Single injection of 1 ml fluid toxoid	Intramuscularly	15	15	7	2.7
		Subcutaneously	14	13	5	2.8
2.	Fractional injection of 1 ml fluid toxoid (9 injections every 3 hours, first, 0.2 ml, remainder, 0.1 ml	Intramuscularly	14	13	1	4.0
		Subcutaneously	15	13	1	3.0
3	Single injection of 1 ml adsorbed toxoid	Intramuscularly	15	0	0	–
		Subcutaneously	15	0	0	–
4	Control (no toxoid injected)	–	15	15	15	2.4

As is seen from Table 1, in which the results of the experiment are presented, a single injection of fluid toxoid showed itself to be the least effective; fractional injection of toxoid gave better results. Immunization with adsorbed toxoid showed itself to be the most effective– in this case the animals not only did not succumb, but did not show signs of tetanus intoxication. From the table it is also evident that essentially no difference in effectiveness of immunization was observed between subcutaneous and intramuscular injection.

Therefore, even the first experiments employing the different programs of immunization referred to, using crude tetanus toxoid, showed that up to the 12th day the most long-lasting immunity is produced by immunization with adsorbed toxoid.

In the following part of the work it was attempted to throw light on the question of the rapidity of development of immunity after the first injection. For comparison two basic schedules of immunization were chosen

* We take this occasion to than Prof. G. V. Vygodchikov and coworkers of the Anaerobic Division of the IEM AMN USSR for presenting us with the preparations referred to.

which had given positive results in the preceding experiment: fractional injection of native toxoid of series No. 336 according to the optimal schedule of the rapid method (9 times per day at intervals of 3 hours), and a single injection of the same dose – 1 ml containing 65 EU of toxoid, adsorbed on aluminum hydroxide (5 mg $Al_2(O)_3$ to 1 ml toxoid; complete adsorption of toxoid). As before, we used subcutaneous and intramuscular injection of the preparation. We tested the resistance of the animals to 2.5 MLD of toxin at various times after immunization.

As can be seen from Figure 1, with fractional injection of tetanus toxoid an increase in resistance in part of the rats was recorded from the 8th day after immunization. However in this case all animals suffered from tetanus and more than half of them succumbed in a relatively short time. By the 10th day the resistance had increased somewhat, and on challenge on the 12th day only single rats succumbed, but all suffered from tetanus.

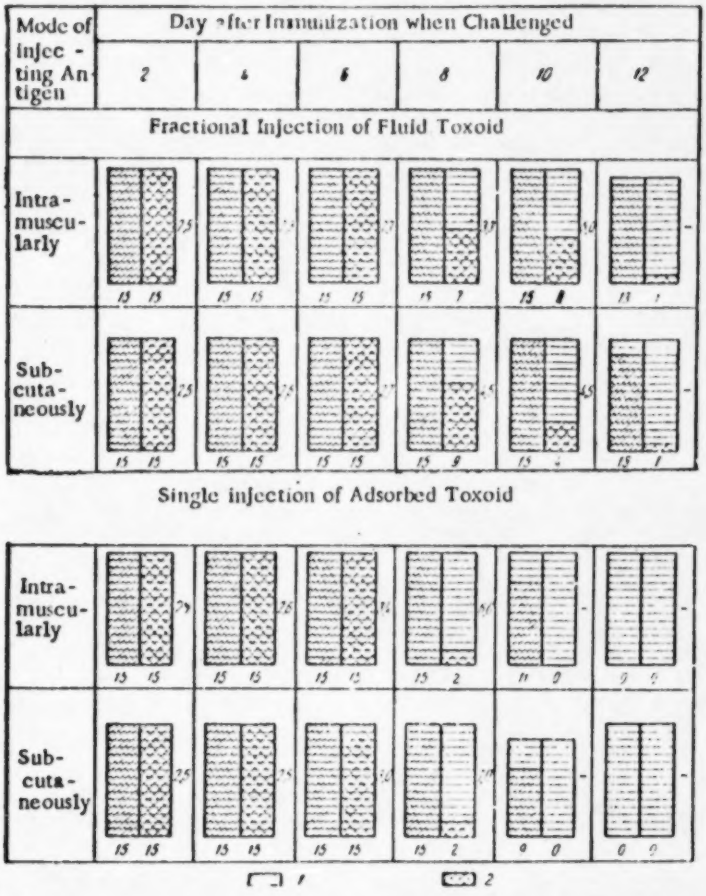

Fig. 1. Rapidity of development of immunity after fractional injection of fluid and single injection of adsorbed tetanus toxoid.
Meaning of Symbols: each division of a bar corresponds to 1 animal; 1) animals with toxic reaction, 2) animals succumbing. Unshaded divisions, unaffected animals. Figures beneath bars, number of animals dying and with toxic reaction. Figures beside bars mean life of animals which succumbed (in days).

Following immunization with adsorbed toxoid the resistance of the animals was already clearly expressed (only single rats succumbed, on the 10th day not a single rat succumbed and several animals did not suffer from tetanus, and on the 12th day the resistance was such that all animals remained unaffected after receiving the toxin.

Thus after immunization by fractional injection of toxoid the resistance of the animals develops significantly more slowly than following immunization by a single injection of adsorbed toxoid, and on the 12th day it is approximately equal to that observed on the 8th day in animals receiving the adsorbed toxoid. If we judge by the degree of immunity, the speed of its development following immunization with adsorbed toxoid under the above-mentioned conditions of the experiment was about $1^1/_2$ higher than following fractional injection of the toxoid.

From Figure 1 it can also be seen that there is no substantial difference in the effectiveness of immunization, in its degree or in the time of its development, following subcutaneous and intramuscular injection of the toxoid.

Further investigations showed that the development of resistance in the animals comparatively soon after immunization with adsorbed toxoid was explained by the development of active immunity with antitetanus antitoxin in the blood. On the 10th day after the injection of the toxoid there could be demonstrated to be present in the blood of the rats antitoxin with a titer of the order of 0.025—0.05 AU, and on the 12th day, 0.1 AU. At the same time following immunization by fractional injection even on the 12th day the titer of antitoxin did not exceed 0.005 AU. Therefore, even in respect to the actual titer of antitoxin, immunization with adsorbed toxoid is better than immunization by fractional injection of fluid toxoid; afterwards the time of appearance of antitoxin in the blood coincides with the above-mentioned time of development of resistance in the animals.

Thus the material accumulated attests the primary immunization against tetanus with tetanus toxoid adsorbed on aluminum hydroxide is definitely more effective than other methods of immunization tried, among them immunization by fractional injection of toxoid, which was characterized in its time as "rapid immunization". Considering the data it is clear that this designation for fractional injection of toxoid, cannot be justified since immunization with adsorbed toxoid builds up not only a more pronounced, but an earlier immunity. Recently G. V. Vygodchikov and coworkers [4] read a communication on the possibility of producing immunity in rabbits a relatively short time after the initial immunization with adsorbed tetanus toxoid.

Having obtained the data presented from a study of immunization against tetanus, we carried out analogous investigations on immunization against gas gangrene. Various schedules of immunization were investigation, among them fractional injection, both of crude and of purified concentrated perfringens toxoid.

In Table 2 are presented the data on immunization of rats with crude toxoid. As can be seen from the table, the best results were obtained when adsorbed toxoid was used.

However the crude toxoid used contained a very moderate number of combining units (about 2 CU in 1 ml), and therefore, before drawing final conclusions, the following experiments were set up, using purified concentrated toxoid, in 1 ml of which there was about 40 CU.

As can be seen from Table 3, in which the results of these experiments are presented, of all the methods of immunization, only the use of adsorbed toxoid stimulated the production of clear-cut immunity. Fractional injection of fluid toxoid, whether at intervals of 1 hour or at intervals of 3 hours, proved completely ineffective. As in the case of immunization against tetanus, it was impossible to note any definite difference in the grade of immunity following subcutaneous and intramuscular injection of adsorbed toxoid.

Thus, in immunization against gas gangrene, even more clearly than in immunization against tetanus, the use of adsorbed toxoid ranks before the other methods of immunization tried, among these, before, the method of fractional injection of toxoid showed itself to be completely ineffective under the conditions of the experiment.

In the following series of experiment there was studied the rapidity of development of immunity to perfringens toxin after a single immunization with adsorbed toxoid. We tested the resistance of the animals in the early stages, beginning with the 6th day after the intramuscular injection of 0.5 ml of purified concentrated toxoid (20 CU), adsorbed on aluminum hydroxide (5 mg Al_2O_3 to 1 ml toxoid).

As can be seen from from Figure 2, pronounced resistance of the animals was observed even on the 8th day after immunization. We cannot refrain from calling attention to the fact that the results of these experiments agree with the data of the analogous experiments on immunization against tetanus. There and here we observe the abrupt development of pronounced resistance on the 8th day after immunization with adsorbed toxoid. These data allow one to suppose that in both cases under the conditions of the experiments described we are evidently encountering one and the same principle of immunogenesis.

TABLE 2

Effectiveness of Immunization with Crude Toxoid of Cl. perfringens Employed in Different Ways

No. of group	Schedule of Immunization	Mode of injection of Antigen	No. of experimental animals	Died
1	Single injection of 2.0 ml fluid toxoid	Intramuscularly	15	15
2	Three 1.0 ml injections of fluid toxoids at intervals of 10 days	»	15	12
3	Three 1.0 ml injections of fluid toxoids at intervals of 5 days	»	15	9
4	Fractional injection of 2.0 ml fluid toxoid (5 injections at intervals of ½ hour, First injection 0.8 ml. Others 0.3 ml each.	»	10	10
5	Fractional injection of 2.0 ml fluid toxoid (10 injections of 0.2 ml each at intervals of 3 hours)	»	15	15
6	Fractional injection of 2.0 ml fluid toxoid (10 injections of 0.2 ml each at intervals of 1 hour)	»	15	15
7	Single injection of 1.0 ml adsorbed toxoid	»	15	8
8	Single injection of 1.0 ml of Adsorbed toxoid	»	15	0
9	Control	—	15	15

In the last series of experiments there were compared the effectiveness of the immunizing action of purified and crude toxoid, given in the same dose (in CU), but according to different immunization schedules. These experiments were designed to obtain some data which might bring us closer to an understanding of the mechanism of action of toxoid under the conditions studied.

The experiments were set up with tetanus toxoid. The dose chosen contained 60 CU. We did not dilute the crude toxoid corresponding to this, but diluted the purified and concentrated toxoid 25 times. In all cases we injected the toxoid intramuscularly.

As can be seen from Fig. 3, the crude toxoid, used in the same dose as the purified, whether as a single injection or as fractional injection, shows a more pronounced immunizing effect. This is reflected in the numbers of animals suffering from tetanus and the numbers of those which died following the injection of toxin at various times after immunization (8th day, 10th day, 12th day) and also in the average survival time of the animals which succumbed. When the same toxoid was used, but adsorbed on aluminum hydroxide (the degree of adsorption was complete in both cases), the above-mentioned differences in immunizing effect were not observed.

Analogous data on the diminution in immunizing properties of toxoid on purification were obtained previously by other authors [9, 8, 2]. On the basis of this Ramon [14] did not recommend purified toxoid for practical purposes.

TABLE 3

Effectiveness of Immunization with Purified Concentrated Perfringens Toxoid Employed in Various Ways

No. of group	Schedule of immunization	Dose of Antigen in ml	Mode of injection of Antigen	No. of experim. animals	Died
		Liquid toxoid			
1	Single injection	1	Intramuscularly	20	20
			Subcutaneously	20	19
2	Fractional injection (5 injections of 0,2 ml each at intervals of 1 hour)	1	Intramuscularly	20	18
			Subcutaneously	20	18
3	Fractional injection (10 injections of 0,1 ml each at intervals of 3 hours)	1	Intramuscularly	15	15
		Absorbed toxoid			
4	Single injection	0,25	Intramuscularly	20	4
			Subcutaneously	20	2
5	Single injection	0,5	Intramuscularly	15	1
			Subcutaneously	15	1
6	Single injection	1	Intramuscularly	20	1
			Subcutaneously	20	1
7	Control	—	—	15	15

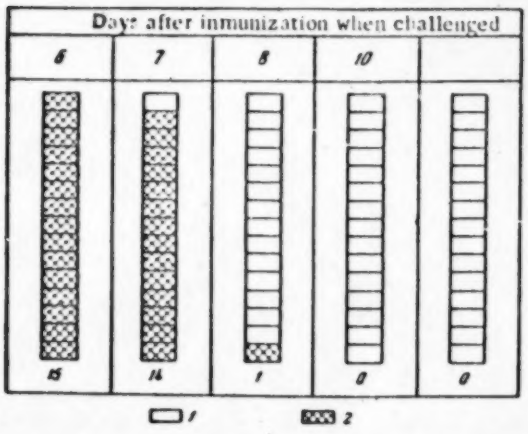

Fig. 2. Resistance of animals to perfringens toxin at different times after immunization with adsorbed toxoid.
Meaning of Symbols: each division of the bars corresponds to 1 animal; 1) survived, 2) died. Figures beneath bars, numbers of animals dying.

Consequently, crude toxoid contains a series of substances which act not only as ballast but serve also to intensify immunogenesis. It is possible that in crude toxoid, along with substances which really act merely as ballast and deleterious substances, there are substances promoting the immunogenic action.

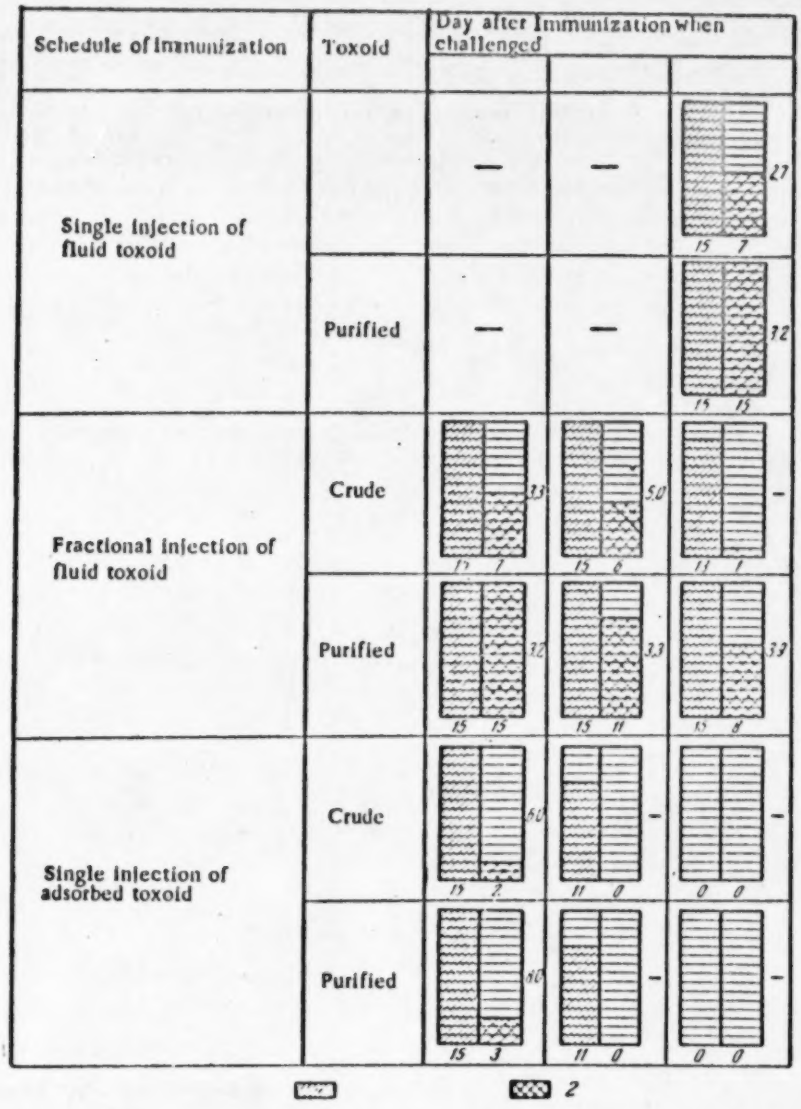

Fig. 3. Effectiveness of immunization against tetanus, using crude and purified toxoid in various ways.
Meaning of Symbols: same as in Fig. 1.

The data obtained can also be interpreted from the point of view of the significance of local reactivity of altered tissues and their lymphatic apparatus as influenced by the antigen. Various impurities in the toxoid may intensify the suggested inflammatory change and thus promote immunogenesis. It is evident that the better immunizing effect of repeated fractional injection of tetanus toxoid into the same place (what is called rapid immunization), compared with a single injection, depends to a significant extent upon the development of a local inflammatory reaction. It is just in this direction that Ramon's process turns one's thinking. Such a mechanism also has definite significance in the use of adsorbed preparations, which always call forth an inflammatory reaction

at the point of injection. However not all inflammatory reactions, nor inflammation in general, least of all the physiological component of an inflammatory reaction, play a positive role. Thus, on the fractional injection of gangrene toxoid, which because of the strength of the residual toxicity provokes an extensive and severe destructive inflammatory change, immunity, as already shown, does not arise. On the fractional injection of tetanus toxoid the local inflammatory change is significantly more weakly expressed, while immunity is more pronounced. Finally, when adsorbed toxoid is used the inflammatory changes may be expressed even more weakly, but the immunity proves to be more pronounced. Analogous observations were made by other authors also, studying local changes resulting from various methods of immunization, including adsorbed toxoid [3, 2]. Ramon and coworkers [13, 16] also showed that not all inflammation promotes immunogenesis.

It occurs to us that the significance of adsorption is not restricted to the fact that it brings about slower introduction of the antigen (and connected with this the optimal conditions for the action of the over-all mechanism of the antigenic action, which has, as is well-known, an important significance for the stimulation of immunogenesis [5, 6]), and not restricted to the fact that adsorbant as an additional stimulant promote the development of reactive changes, but evidently also resides in the fact that adsorbants alter the physicochemical state of the antigen, its corpuscular structure. Similar conceptions have been expressed by other authors [17, 12]. It is possible that some of the impurities in crude toxoid may also play an analogous role.

SUMMARY

Experiments with white rats showed that a single injection of purified or crude tetanus toxoid adsorbed on aluminum hydroxide produces earlier immunity than fluid toxoid injected in various ways, including fractional injection at intervals in the course of a day.

Immunization against Cl. perfringens (Cl. welchii) was effective only when adsorbed toxoid was used, resistance developing relatively early (on the eighth day). Other methods of immunization, either with crude or purified toxoid, were not effective. The rapidity of development and the degree of immunity, both to tetanus and to gangrene, did not depend upon whether the antigens were injected intramuscularly or subcutaneously.

Purified tetanus toxoid without adsorbant was less effective than the same dose of crude toxoid.

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EXPERIMENTAL BIOLOGY

THE EFFECT OF FUNCTIONAL LOADING ON LIVER REGENERATION IN RATS

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Although the morphological changes which occur during mamalian liver regeneration have been well studied, the functional condition of the liver during the regeneration process and the influence of the liver function on liver regeneration have not yet been sufficiently investigated. As well as being of general biological interest, these questions are extremely important to medicine, especially surgery.

With the question of how the functional condition of the liver influences the intensity of regeneration, we started from a proposition expressed by M. A. Vorontsova and L. D. Liozner [1], according to which, there is a close connection between physiological and reparative regeneration. Processes restoring the structural elements of each organ, i.e., physiological regeneration, occur as a result of the organ's functional operation. These regenerative processes, which occur in the normal conditions of the animal's life, constitute the original background on which the processes of reparative regeneration occur. One can therefore assume that intensification of the organic function, which causes intensification of physiological regeneration, would also affect reparative regeneration. The almost complete lack of data covering this important aspect of research caused us to conduct experiments which would throw some light on this relationship. How different degrees of functional loading affected liver regeneration was a question that interested us particularly. For this purpose, we made experimental tests to find whether the bile-secreting function of the liver increased or decreased by using choleretic substances and selecting a corresponding diet. Although there are a series of works in the literature studying the influence of different diets on liver regeneration in mammals (Opie and Alford [6], Davis and Whipple [4], Moise and Smith [5], Brues, Drury and Brues [3], and Podzolkov [2]), the data in these works is rather contradictory. Moreover, these studies did not treat the question of how liver function influences liver regeneration and thus did not have direct bearing on the problem which interested us.

Our experiments were conducted on white rats.

The plan of the experimental setup is illustrated in Table 1.

The experimental animals were divided into three groups. The first group of rats (30 animals) were kept on a choleretic diet. The rats were given fresh dog's bile daily as the choleretic substance. In addition, the diet consisted principally of food substances causing strong bilification.

The second group of rats (30 animals) received a sparing bilification diet, which principally consisted of food substances causing weak bilification.

The third group (30 animals) was fed a normal diet.

After being fed the indicated diets for 3 days, the animals of all three groups were operated upon to remove the large, left liver lobe. The average weight of the extirpated lobe was 2 g. Since the average weight of the

liver was 9 g, approximately $^1/_6$–$^1/_4$ of the liver was removed.

After the operation, the animals continued on their respective diets (choleretic, sparing and normal).

TABLE 1

Time of fixation	Experimental rats			Control rats		
	Diet					
	Choleretic	Sparing	Normal	Choleretic	Sparing	Normal
Day of operation						10
1 day after operation	10	10	10			
7 days after operation	10	10	10			
14 days after operation . . .	10	10	10	10	10	10
Total number of rats	30	30	30	10	10	20

The animals were killed 1 hour, 1 week and 2 weeks after the operation (10 rats from each group were killed at each of these times).

The material was fixed in Carnoy's, Helly's and Zenker's fluids and in formalin. The material was processed for glycogen and fat, and the preparation was stained with hematoxylin-eosin, by Mallory's method and by Van Gieson's method.

The group of rats which had been fed a normal diet, but had also had the liver partially removed, served as the control in the examination of the effect of the choleretic and sparing diets on liver regeneration.

In addition, 10 rats were killed before the operation (initial control) and 10 rats at the end of the experiment (final control).

Supplementary controls were used in order to distinguish the effects of the diets on the liver condition and on the course of the regeneration process in the liver; rats which had not had the operation but had been fed the choleretic diet (10 animals) served as the control for the choleretic diet, and rats which had not had the operation but had been fed the sparing diet (10 animals) served as the control for the sparing diet. The animals of these two controls groups were killed at the same time as the experimental rats, 2 weeks after the operation.

We determined the weight of the rats before the operation, the weight of the extirpated portion of the liver, the weight of the rat when killed and the weight of the liver at the time the rat was killed. The relative weight of the extirpated portion of the liver to the weight of the body and the relative weight of the liver to the weight of the body were also computed. The material was processed statistically. The data obtained is summarized in Table 2 and graphically presented in Figures 1-3.

As Table 2 shows, the weight of the remaining part of the liver sharply increased in the rats of all groups as early as a day after the liver lobe had been resected. Although a rather large number of mitoses was observed in the hepatic cells at this time, it did not seem possible after such a short time that the liver weight had increased only because of the increased number of hepatic cells. It seemed more likely that this liver weight increase was connected with the reaction processes. At this time, histological study showed, along with the necrotic changes in the injured surface region of the liver, a profuse infiltration of large drops of fat, most clearly expressed around the periphery of the small lobes and change in glycogen distribution, which was shown by the fact that while some of the hepatic cells had lost their glycogen, others, which did contain it, were almost completely clogged with it.

One week after the operation, the weight of the regenerating liver was substantially different in the different groups of experimental rats. For example, in the rats kept on a choleretic diet, the weight of the regenerating liver exceeded the weight of the normal liver, while, in the animals fed the control diet, the weight of the liver

was less than the weight of the liver a day after the operation. The liver weight in the rats fed the sparing diet was about the same as it had been one day after the operation. The decrease in the liver weight 1 week after the operation, as compared with the first days after the operation, which was observed in the rats kept on the control diet confirmed our proposition that the increase in the liver weight the first day after the operation was due to a reaction. That liver regeneration continued during this period was indicated by the still rather large number of mitoses, while the inflammatory phenomena connected with the trauma were already abating. The drops of fat infiltrating the hepatic cells became small and the infiltration less marked. Staining for glycogen showed a picture approximating that in the control animals.

TABLE 2

Liver Regeneration in Experimental and Control Rats

No. of test	Series	Average postop. wt. of rats	Average wt. of rats when killed (in g)	Average wt. liver when rat killed (in g)	Average rel. wt. liver to wt. body when killed (in %)
1	Initial control		201	9 4	4 6
2	Experiment, normal diet				
	1 day after operation . . .	197	193	8.0	4.5
	1 week after operation . .	197	193	7.2	3.6
	2 weeks after operation . .	196	189	9.5	5.0
3	Experiment, sparing diet				
	1 day after operation . . .	203	197	8.3	4.12
	1 week after operation . . .	204	205	8.3	4.0
	2 weeks after operation . . .	194	202	9.8	4.8
4	Control, Sparing diet		195	8.8	4.5
5	Experiment, chloretic diet				
	1 day after operation . . .	195	186	7.8	4.1
	1 week after operation . . .	202	173	9.2	5.2
	2 weeks after operation . . .	203	206	10.4	5.0
6	Control, chloretic diet . . .		206	9.3	4 5
7	Final control		204	8.6	4.2

Two weeks after the operation, the weight of the regenerating liver in the rats fed the control and sparing diets became slightly greater than the liver weight in the rats of the intial and final controls. After the same period, only the absolute weight of the liver had increased in the rats fed the choleretic diet. This is explained by the fact that the whole body of the rat had grown rather considerably by the second week after the operation.

Two weeks after the operation, the weight of the regenerating liver in all three groups of the rats exceeded the original weight.

During the period of the greatest increase in the weight of the regenerating liver, the body weight of the rats decreased; this was especially evident in the rats fed the choleretic diet.

One can assume from the results of the histological examinations made of the regenerating liver of the rats that the restoration is realized by regenerative hypertrophy.

The intense regeneration in the liver of rats fed the choleretic diet is evidently connected with the more rapid divisions of the cells; we cannot, however, prove this completely satisfactorily since there was no detailed study of hepatic cell mitotic activity in our work.

Our data, then, showed that the functional condition of the liver does affect liver regeneration. Enhanced functional load created by feeding the rats the choleretic diet caused the weight of the regenerating liver to exceed the weight of the normal liver as soon as the end of the first week after the operation. Therefore, liver regeneration is intensified by an enhanced functional load. That intense, regenerative hypertrophy did in fact occur was shown by the increase in the liver weight and by the fact that the regenerated liver had a normal structure.

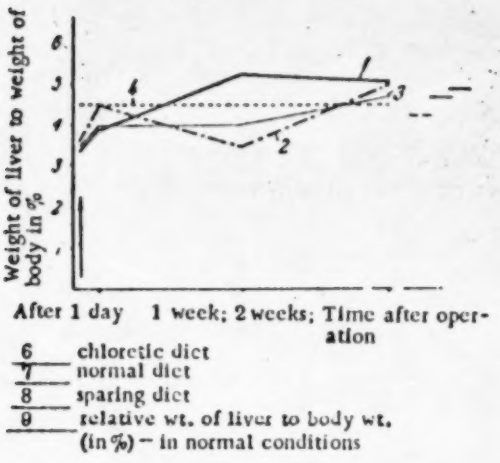

Fig. 1. Change in absolute liver weight of rats during the regeneration process. 1) choleretic diet; 2) normal diet; 3) sparing diet; 4) average weight of liver in the control rats.

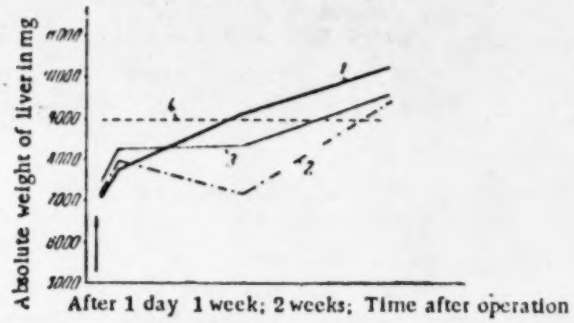

Fig. 2. Change in relative weight of rat's liver during regeneration. 1) choleretic diet; 2) normal diet; 3) sparing diet; 4) relative weight of liver to body weight in the norm (in %).

The latter proves that the liver weight increase observed was not caused by any pathological processes. Since in no case did we find abnormal growths of hepatic cells, in our experiments, liver regeneration was apparently caused both by hepatic cell divisions and by organization in the lobules. The cells of the bile ducts did not take any important part in the regeneration process. Only in two cases did we observe abundant growth, similar to adenomatous nodes, in the bile ducts.

SUMMARY

Ninety albino rats with the large left liver lobe extirpated were sustained on various diets (control, sparing and choleretic). In rats fed a choleretic diet regeneration of the liver was most complete. In one week after the operation the initial relative weight of the liver was already restored, while in the other groups restoration took place only after two weeks.

Restoration of the weight of the liver was due to an increase in the number of liver cells.

Thus, an enhanced functional load of the liver stimulates its regeneration.

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FUNCTIONAL AND STRUCTURAL CHANGES IN THE OVARIES OF RATS FED A WELL-BALANCED DIET AFTER BEING KEPT ON A PROTEIN-DEFICIENT DIET

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It has recently been established that the normal function of the ovaries is easily disturbed by different unfavorable influences on the body—by the toxic effect of various poisons [1, 4], during infectious diseases [2, 8], by the action of roentgen rays [9, 3], or by deficient or unbalanced diets.

Chronic starvation (partial or protein) delays sexual maturation in young animals [6, 7, 10] and suspends sexual development in adult animals [11].

Some authors have observed atresia intensification in the ovaries of starved animals and considerable connective tissue growth [7, 12, 13] as well as functional disturbance of the gonads.

However, the dyanmics of the destructive changes occurring in the ovary during long protein starvation and the question of whether the disturbed function and structure of the ovary can be restored especially have not yet been sufficiently investigated. In an earlier paper [5], we established that long protein starvation causes the ovaries to decrease in weight and their function and structure to be disturbed.

This work presents data concerning the changes observed in the ovaries of animals which were fed a balanced diet after long protein starvation.

EXPERIMENTAL METHODS

The experimental animals were fed synthetic diets—the control diet, with a 19.5% protein content and the experimental, with a 1.5% protein content. The fat, vitamin and mineral contents were the same in both diets.

The experiments were done on sexually immature (45-55 g) and sexually mature (140-156 g) rats.

The immature rats were fed a protein deficient diet for 45-64 days, during which time they lost 29-37% in weight. The mature rats were kept on the diet with the 1.5% protein content for 90-135 days. During this period, their weight decreased 35-53%.

After long protein starvation, some of the animals from each group were transferred to the control diet (19.5% protein). The immature rats were sacrificed on the 4th, 7th, 14th and 17th days of the balanced diet and the mature, on the 14th and 50th days.

All of the animals were sacrificed during the inactive phase of the sexual cycle. The ovaries were weighed in torsion scales and fixed in Zenker-formol fluid. The material was imbedded in paraffin. A series of sections 8 microns thick were prepared. The preparations were stained with hematoxylin-eosin. The ovarian structural components were counted in every fifth section (primordial follicles, follicles at different stages of maturity,

atretic follicles, corpora lutea and follicles with destroyed oocytes).

The results of the computations and the data on the relative weight of the ovaries were processed statistically.

EXPERIMENTAL RESULTS

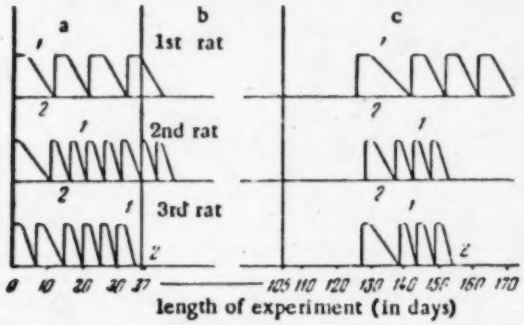
The absolute weight of the ovaries in all the rats fed the control diet after long protein starvation was greater than the weight of the ovaries in the starved animals (Tables 1 and 2).

The relative weight of the ovaries in the immature rats increased during the first 4-7 days of the balanced diet, then decreased and became even somewhat less than the relative weight of the gonad in the animals before protein starvation (Table 1). During this period, apparently, the growth processes proceeded very vigorously in the young animals, and the growth of the body considerably outstripped the growth of the ovary.

In the mature rats, depending on how long they had been fed the balanced diet, a progressive increase of both the absolute and relative weight of the ovaries was observed (Table 2).

In the immature rat group, the animals had still not reached sexual maturity by the 17th day of observation. After protein starvation, a few of the immature rats were left on the control diet for a 4-month period. After this period, not only did estrus appear, but the rats were capable of propagation.

In the mature rats fed the control diet after long protein starvation, the normal function of the ovaries was not restored immediately: the first estrus appeared on the 22nd-24th days. The inactive period between the first and the second estrus was longer than the ensuing ones, being 7 days in some rats, 12 in others. Then the normal sexual cycle was restored and repeated at the same intervals as in the animals kept on the control diet (see Figure)..



Sexual cycle of white rats under different conditions of protein feeding. a) control (diet with 19.5% protein); b) experiment (diet with 1.5% protein); c) restoration (diet with 19.5% protein); 1 – estrus; 2– diestrus.

The results obtained from counting the structural components in the ovaries of the immature rats put on a balanced diet after protein starvation are given in Table 1. For the first 4 days of the balanced diet, there were no substantial changes in the number of ovarian structural components. The primordial follicles and follicles with destroyed oocytes were exceptions, as there were slightly more of them per section in the starved animals than in the rats on the control diet; the difference in the number of maturing and atretic follicles were not verified statistically.

The average number of maturing follicles did not change noticeably when the animals were kept on the control diet for a longer period (14-17 days). After 14-17 days of the balanced diet, the decreased number of atretic follicles and follicles with destroyed oocytes indicated that atresia was less intense. We were especially interested by the fact that the number of primordial follicles was greater in the ovaries of rats kept 14-17 days on the balanced diet than in the ovaries of rats kept only 4 days on this diet.

TABLE 1

Change in Ovary Weight and Structure in Immature Rats Fed the Control Diet after Protein Starvation

Animal group	No. of rats	Absolute wt. ovaries in mg.	Relative wt. of ovaries in % ± m.	Average No. of structural components per section ± m				
				Primordial follicles	Maturing follicles	Corpora lutea	Follicles with destroyed oocytes	Atretic follicles
Rats kept on experimental diet for 64 days	6	4.5	14.1±0.44	12.7±0.42 (28.5%)	9.7±1.07 (21.2%)	—	3.8±0.09 (8.2%)	19.4±1.32 (42.1%)
Rats kept on control diet for 4 days after starvation	5	7.5	16.3±0.94	7.2±1.20 (19.0%)	7.8±0.79 (20.8%)	—	2.6±0.32 (6.8%)	20.9±2.60 (53.4%)
Rats kept on control diet for 14-17 days after starvation	5	8.4	12.9±0.42	12.1±2.13 (32.6%)	9.0±0.59 (25.5%)	—	1.8±0.23 (5.0%)	13.7±2.34 (36.9%)

TABLE 2

Change in Ovary Weight and Structure in Mature Rats Fed the Control Diet after Protein Starvation

Animal group	No. of rats	Absolute wt. of ovaries in mg.	Relative wt. of ovaries in % ± m.	Average quantity of structural components per section ± m				
				Primordial follicles	Maturing follicles	Corpora lutea	Follicles with destroyed oocytes	Atretic follicles
Rats kept on experimental diet for 135 days	5	16.6	20.7±1.28	7.7±0.44 (17.9%)	2.8±0.32 (6.4%)	6.4±1.44 (14.1%)	4.2±1.43 (8.7%)	23.6±2.06 (52.9%)
Rats kept on control diet for 14 days after starvation	3	34.2	24.7±3.10	15.4±6.47 (34.1%)	5.4±0.30 (12.4%)	8.6±2.82 (20.1%)	1.0±1.52 (2.1%)	13.6±3.41 (31.3%)
Rats kept on control diet for 50 days after starvation	4	47.8	26.4±1.50	5.4±0.32 (15.4%)	10.1±1.01 (28.5%)	9.3±0.60 (26.3%)	0.9±0.10 (2.4%)	9.7±0.47 (27.4%)

New primordial follicles evidently form in the ovaries of animals put on the control diet after long protein starvation.

In the mature animals, on the 14th day after their transfer to the protein rich diet, no statistically verifiable differences in the average number of primordial follicles and corpora lutea could be discovered, as compared with that found in the ovaries of the starved rats (Table 2). The increased number of maturing follicles could be connected either with the decreased number of follicles undergoing regressive development or with the intensification of their differentiation from the primordial follicles. The decreased number of atretic follicles and particularly of the follicles with destroyed oocytes indicates the diminished intensity of atresia in the ovaries of the animals after 14 days of the balanced diet.

After 50 days of the balanced diet, there were considerably less primordial and atretic follicles, as well as less follicles with destroyed oocytes, in the rat ovaries than in those of the rats fed the protein deficient diet. The average amount of maturing follicles, however, increased (see Table 2). Consequently, after 50 days of the balanced diet, the differentiation process became intensified in the ovaries of the animals.

The change in the interrelation of structural components in the ovaries of the mature rats, after their transfer to the balanced diet, was about the same as in the immature rats. In both cases, atresia lessened, and the absolute number of primordial follicles increased. The latter was evidenced by the fact that the amount of primordial follicles per section was approximately the same as in the ovaries of the starved rats, whereas the weight and size of the ovaries, and consequently the number of ovarian sections, was considerably greater in the rats kept on the control diet.

On the basis of the research conducted, we concluded that the processes of growth and follicular differentiation are resumed in the ovaries of rats transferred to a balanced diet after long protein starvation; in mature rats, the follicles mature through ovulation, and new corpora lutea appear. The normal function and structure of the ovaries is restored.

SUMMARY

Sexually mature and immature rats kept for a long time on a diet deficient in protein (1.5% protein) were fed a control diet of 19.5% protein content. The functions, relative and absolute weight of the ovaries were restored. The microscopic structure of the organ was restored, likewise the growth processes and follicular differentiation were resumed. In comparison with the period of dificiency the number of oocytes markedly increased.

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AGE CHANGES IN THE LIPOFUSCIN CONTENT OF THE HUMAN HEART MUSCLE

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The question of the role and deposition conditions of lipofuscin has not yet been solved.

The most widespread opinion is that regarding lipfuscin as a typical sign of aging [3, 4, 8], as the "slag" of cell metabolism [1, 8]. Pathologicoanatomists, as Vail [2] observed, often relate brown pigmentation of organs to dystrophic changes. On the other hand, some authors [5, 6, 11] consider lipofuscin to be a substance or structure of functional-physiological importance. The necessity of reexamining the hypothesis that lipfuscin is an expression of body involution ("old age pigment," "depreciation pigment") has recently been emphasized in a series of works [2, 6].

A knowledge of the age dynamics of pigmentation, which has certainly not been sufficiently investigated yet, is needed to evaluate the role of lipofuscin. The majority of the existing works [6, 7] do not give a quantitative analysis of the age changes in pigmentation and do not show the procedural features of the processes during the successive periods of individual development. The results of researches [8-10] using the quantitative method are very contradictory.

The purpose of this work was to make the existing data on the age dynamics of brown myocardial pigmentation more exact by using an exact method of quantitative lipofuscin determination on the material and processing the results statistically.

EXPERIMENTAL METHODS

To examine the age dynamics of myocardial pigmentation, we examined 100 hearts from human cadavers of different ages ranging from 28-week old embryos to 82-year old people. All the material was divided into 10-year age groups: from 0 to 9 years, 5 cases; from 10 to 19, 6 cases; from 20 to 29 years, 21 cases; from 30 to 39 years, 18 cases, from 40 to 49 years, 18 cases; from 50 to 59 years, 12 cases; from 60 to 69 years, 10 cases and from 70 to 79 years and over, 10 cases.

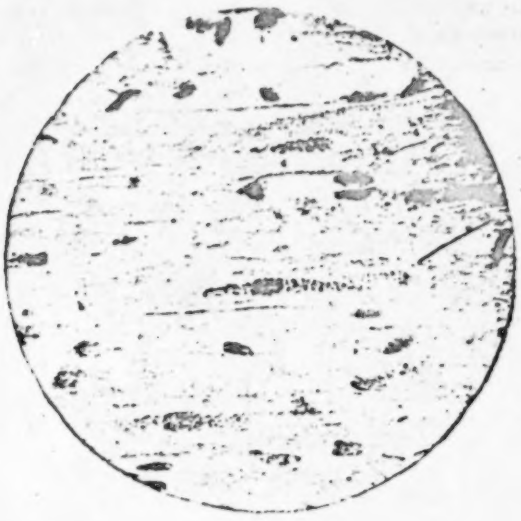
The lipofuscin content was determined by planometric measurement.

The pigment content in the myocardium was determined in the following manner. From the anterior wall of the left myocardial ventricle, sections were made on a freezing microtome. The stains used were Sudan III and hematoxylin. The sections were imbedded in glycerinated gelatine. With the aid of a drawing apparatus, drawings were made of the nuclear contours from the preparations and of the pigment adjoining them, which, in the myocardium, was deposited like clearly-defined cones at each pole of the nucleus (see Figure). From each heart, the contours of 80 pigment cones and of 40 nuclei were drawn. The area of the drawn cones was measured by a planimeter. Then the average area of the perinuclear, pigment cones in each of the experimental myocardia

and the average area of lipofuscin deposition for each age group were determined.

The numerical data obtained were processed statistically to verify the established age differences.

The advantage of this method over those used in previous works (visual comparison of lipofuscin content in the sections [3], correlation of number of strongly and weakly pigmented cells [12], linear measurements [8, 9]) was that it made it possible to measure the area occupied by the lipofuscin deposits and therefore to obtain exact numerical data on the degree of pigmentation in each individual case and in the whole age group.



Heart Muscle with Lipofuscin Deposits

EXPERIMENTAL RESULTS

The following was established from the research conducted. There was no pigment in children up to 9 years old; pigment was found in four out of the six hearts examined in the age group 10-19 years, but not in hearts less than 12 years old; pigment was found in every case in the other age groups. Therefore, lipofuscin was discovered in the myocardium of each cadaver over 12 years old, both in those which had died from trauma and in those which had died from various diseases. Since most of the cadavers had been generally healthy people, this confirms that the pigmentation is not caused by pathological factors and that it should rather be considered a general physiological rule, since lipofuscin is present in the myocardium after the beginning of the second decade.

The lipofuscin content was determined in the hearts of the different age groups in order to evaluate the subsequent course of the pigmentation process (Table 1).

TABLE 1

Change in Lipofuscin Content in People of Different Ages

Group	I	II	III	IV	V	VI	VII	VIII
Age (in years)	0—9	10—19	20—29	30—39	40—49	50—59	60—69	70—79
Average area of lipofuscin deposit (in units of planimetric scale)	—	124	200	270	310	317	300	342
Fluctuation limits	—	77—221	117—343	160—339	203—458	138—501	180—410	204—447

As Table 1 shows, the myocardial lipofuscin content increases with age, attaining the maximum during old age. Great individual variations were observed within each age group, often exceeding the average pigment content in the succeeding age groups.

In order to have a complete picture of the age dynamics of pigmentation, the intensity of pigment accumulation at different stages of individual development had to be determined. The absolute increase of the average area of the lipofuscin cones in each age group as compared with the preceding group was used as the index. Comparing the increases (Table 2) showed that the most intense accumulation of lipofuscin occurred during the first years after its appearance. For example, the average pigment area in Age Group II was 124 units when the highest age group's average was 342 units, i.e., Group II was already almost 40% of the average area in Age Group VIII. Therefore, the period from 10-19 years was characterized by intense pigmentation, which was also observed in Age Groups III and IV, while the intensity of lipofuscin deposition sharply decreased in the succeeding, older age groups.

TABLE 2

Intensity of Lipofuscin Deposition at Different Ages

Group	I	II	III	IV	V	VI	VII	VIII
Age (in years)	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79
Increase of lipofuscin area (in units of planimetric scale)	—	124	76	70	40	7	—17	42

The data obtained indicate that the amount of lipofuscin actually increases with age, but that, contrary to present opinion, [3, 8] the greatest intensity of pigment deposition occurs during the first half of life, while pigment deposition decreases during old age.

The amount of pigment increases especially during the first years after its appearance.

The great increase in the amount of lipofuscin during the initial period of individual development and the gradual decrease in pigmentation intensity during the subsequent years would seem to contradict the idea of lipofuscin as an "old age pigment" [3, 4, 8] connected with senile involution. The presence of the pigment cannot be considered an index of old age change, since its accumulation occurs primarily during that period of development when the body is still only approaching morphological and functional maturity. The proposition that lipofuscin is functionally and physiologically important is more probable. The present, widely-held "slag theory" [1, 4, 9], which considers the brown pigment to be the useless waste of cell metabolism, accumulating with age, is the wrong basis on which to study further the properties and role of lipofuscin.

SUMMARY

Ontogenetic dynamics of lipfuscin in the human heart muscle were studied by the method of planimetric measurements. The hearts of one hundred cadavers of the age from 28 weeks up to 82 years were investigated. Beginning with the second decade of life lipofuscin is present on the myocardium of every individual, no matter what the cause of his death was: an occasional trauma or an infectious disease. Deposition of lipofuscin is most intense during the first half of the life span (up to 40 years), most of all, at the beginning of its appearance. Data collected indicate that lipofuscin deposition is a physiological phenomenon and is not caused by aging processes of the organism.

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MORPHOLOGY AND PATHOMORPHOLOGY

THE ADSORPTIVE PROPERTIES OF THE TUNICA VAGINALIS PROPRIA OF THE TESTIS

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The processes of adsorption from serous cavities have long interested clinicians, physiologists and morphologists. Cavity resorption of fluid, crystalloids and colloids, suspensions and normal blood elements – all of these have been the subject of experimental studies and clinical observations. Besides their great theoretical interest, their investigation is very important to the needs of practical medicine. Suffice it to say that the development of practical methods of treating a whole series of diseases, which are attended by an accumulation of transudate and exudate, toxins, bacteria, hemorrhaged blood, etc., in the serous cavities, depends a good deal on accurate information as to the absorptive ability of the corresponding serous membranes in normal and pathological conditions. However, the vast majority of the works treating this subject are concerned with the processes of resorption from the peritoneal, pleural and pericardial cavities. The serous cavity of the scrotum and the tunica vaginalis propria of the testis, which lines this cavity, seem to be beyond these researchers' field of vision.

The only works concerning the absorptive properties of the latter membrane were conducted on clinical material and are only incidental, unorganized observations. The authors injected different substances (salts, stains) into the cavity of the mentioned serous membrane under hydrocele conditions, and then, for definite time intervals, traced their appearance in the urine and other excretions. The results of these studies were highly contradictory. According to some authors [6, 7], resorption from the cavity of the hydrocele sac of the tunica vaginalis propria is extremely intense, while, according to others [2, 5, 8], it is slight or entirely lacking. This difference of opinion seems to be explained by the fact that the researchers worked with different forms of testicular tunica vaginalis propria hydroceles, but did not take the histopathological changes in the membrane or the length of the affection into account. It is even more difficult to form a theory from the results of the observations done on the absorption of substances which differed as to diffusion properties and degree of toxicity. They only indicate that functionally and anatomically damaged testicular membranes are permeable by the given substances.

Of course, in order to solve the question we have mentioned, information is needed as to the absorptive properties of the testicular tunica vaginalis propria under normal physiological conditions. However, to date there has been no work in this field. Researchers have not taken advantage of the procedure of extensive research on animals, although this would seem to be an important stage in discovering the rules regulating the different aspects of resorption from the serous cavity of the human scrotum.

According to the literary data, resorption of serous fluid and various substances suspended or dissolved in the serous fluid from the peritoneal, pleural and pericardial cavities is realized by the vascular bed of the corresponding portions of the serous membrane. It has been established that true and high dispersion colloid solutions are absorbed by both the blood and lymph vessels of the serous membrane, but suspensions and low-dispersion colloids by only the lymph vessels [1, 3].

The purpose of this work was to determine the role of the blood and lymph vessels in the resorption of the above substances from the serous cavity of the testicular tunica vaginalis propria.

EXPERIMENTAL METHODS

We used dogs, for the most part, as the experiment subjects. A few cats and rabbits were also used. The experiments were done on healthy, sexually mature males. Three series of experiments were done: 1) on absorption of true solutions; 2) on absorption of colloid solutions and 3) on absorption of suspensions. In the first series, the indicators used to show the resorption course were light green and orange "G", in the second, trypan blue, and in the third, an India ink or Prussian blue suspension. The staining substances were prepared in a physiological solution of sodium chloride. They were injected into the serous cavity of the scrotum in the following way: while the animals were anesthetized, the skin and the underlying layers of the scrotum as far as the testicular tunica vaginalis propria were opened with a small, longitudinal incision; the tunica vaginalis was lightly pulled away and pierced with a hypodermic needle. The stain was injected into the cavity with minimal pressure exerted on the hypodermic plunger. The volume of fluid injected was usually not more than 1-1.5 cc.

Adsorption of the indicators by the blood vessels of the testicular serous membrane was determined by colorimetry of the urine* and of blood plasma from the peripheral bloodstream. In the lymph vessels, the index of resorption was the presence of the stain in the principal lymph capillaries of the tunica vaginalis propria, in the drainage vessels and in the regional lymph nodes.

The experiments lasted from a few minutes to 24 hours. The animals were killed after fixed intervals. The testicles and the covering layers of the tunica vaginalis propria were fixed in formalin for subsequent histological processing of the material. The regional lymph nodes were also examined.

EXPERIMENTAL RESULTS

In the first series of experiments, the crystalloid solutions of the stains injected into the cavity of the dog's testicular tunica vaginalis propria (8 experiments with 1% solution of light green injected and 5 experiments with a 2% solution of orange "G" injected) began to appear in the urine and blood plasma as early as 8-10 minutes after the beginning of the experiment.

When the serous cavity of the scrotum was opened, the following typical picture of indicator distribution along the surface of the testicular tunica vaginalis propria could be seen by the naked eye: intense, diffuse staining of the parietal layer on the one hand, and hardly noticeable traces of stain in the visceral layer on the other (Fig. 1). When the laminar, total preparations of the testicular serous membrane were examined under a microscope, absorption of the stains on the connective tissue structures of the parietal layer and completely selective blood vessel injection of the latter could be clearly seen. Only a slight staining of the fibrous surface laminae could be observed in the visceral layer. When the preparations were processed with solutions of ammoniacal silver (according to Goier), it was established that the mesothelial sheath was adequately preserved in both layers of the testicular tunica vaginalis propria.

After longer experimental intervals (18-20 minutes or more), another phenomenon was added to the just described morphological picture: the outgoing lymph vessels of the parietal layer of the testicular membrane were observed to be injected and the regional lymph nodes (ln. medialis) to be stained, becoming increasingly more stained as the experimental interval increased.

Therefore, the results of the experiments injecting true solutions of substances into the cavity of the testicular tunica vaginalis propria showed that true solutions are absorbed by both the blood and lymph vessels located in the stratum of the parietal layers of this serous membrane. However, absorption was more intense in the blood vessel bed, which was indicated by the earlier appearance of the indicators in the peripheral blood and urine than in the outgoing lymph vessels.

In the second series of experiments, a colloid solution of trypan blue (1-2%) was injected into the studied serous cavity. Dogs were used as the experimental subjects. Colorimetric analysis, lasting as long as 4 hours, of

*For this, a fistula was induced in the eviscerated bladder, and then the bladder was replaced into the celiac cavity. The bladder contents were removed at fixed intervals with a micropipette.

negative results in all of the 11 experiments. However, we found the stain in the outgoing lymph vessels of the tunica vaginalis propria parietal layer and in the regional lymph nodes (ln. medialis) as early as $1^1/_2$ 2 hours after its injection into the cavity. When the testicular tunica vaginalis propria was exposed, the same phenomenon that appeared in the experiments with crystalloid absorption was observed; i.e., selective absorption, in which, the parietal layer was definitely stained while the visceral layer was almost unchanged in color. When the pellicular preparations were examined under a microscope, it was interesting to observe that, in the parietal layer, the trypan blue was distributed along the edges of the mesothelial cells (Fig. 2). In places, the lymph capillaries were injected with the stain. The light network of canals stood out sharply against the intense blue background of the membrane, showing a "negative" picture of the blood vessels (Fig. 3). In the visceral layer of the testicular serous membrane, one could only find foci of trypan blue distributed along the surface in a few visual fields.

Fig. 1. Tunica vaginalis propria of a dog's testis. 1% solution of light green injected into cavity (experimental interval: 10 minutes). Piece of intensely stained parietal layer sloughed. Visceral layer not stained. Photo. Magnified $1^1/_2$ times in printing.

Fig. 2. Parietal layer of tunica vaginalis propria of a dog's testis. Deposit of trypan blue along the intercellular borders of the mesothelium (duration of experiment: 2 hours). Microphoto. Standard magnification.

Combining the data obtained, we concluded that the chief role in the absorption of colloid solutions (of the trypan blue type) from the testicular tunica vaginalis propria cavity belongs to the lymph vessels in the parietal layer of this membrane. The resorbing function of the blood vessels need hardly be discussed.

In the third series of experiments, 1% suspensions of India ink and Prussian blue were used as the color indicators. A total of 24 experiments were done (10 on dogs, 8 on cats and 6 on rabbits), lasting from a few minutes to 24 hours.

The results of these experiments were identical: in none of the experiments did the suspensions permeate the blood vessel bed of the testicular vaginalis propria, nor did they appear in the lymph vessels or regional lymph nodes draining the studied cavity.

Microscopic analysis of the preparations showed that particles of ink (or Prussian blue) were scattered along the surface of the mesothelium in the visceral layer of the testicular serous membrane. The particles, as a rule, did not penetrate into the underlying layer. In the parietal layer, the suspended particles were mainly distributed along the intercellular borders of the mesothelium. Single granules were found in the cytoplasm of the mesothelial cells. After long experimental intervals (12-24 hours), many histiocytes, phagocytizing the color particles (Fig. 4), were found among the fibrous structures of the parietal layer.

From the data presented above, it is evident that the testicular tunica vaginalis propria and its vascular bed do not directly participate in the resorption of suspensions. This membrane, therefore, is essentially different from

other serous membranes. Corpuscular particles are known to be removed from the peritoneal cavity, the pleural cavity and the pericardial cavity by means of the lymph system. In definite zones of the corresponding membranes, the intraserous lymph vessels are in close contact with the corresponding cavities,* due to the features of the histological structure of the membranes. The resorption of fluids and particles suspended in fluids is provided for in the same way.

Fig. 3. Parietal layer of tunica vaginalis propria of a dog's testis (explanation in text). Microphoto. Standard magnification.

Such relations were not observed in the scrotal serous membrane of the animals we examined. As a rule, the lymph vessels draining that cavity are located in the deep layers of the testicular peritoneal sheath and are separated from its surface by a thick, fibrous wall.

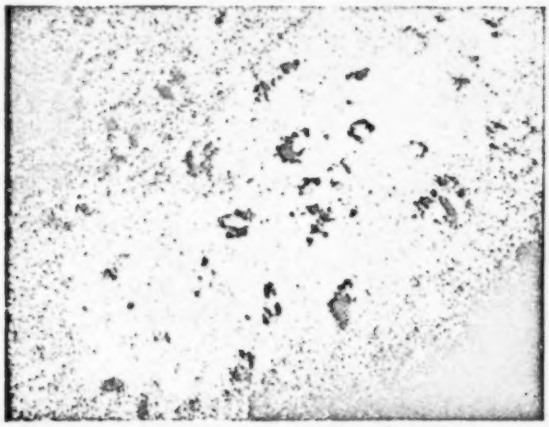

Fig. 4. Parietal layer of tunica vaginalis propria of a dog's testis. Accumulation of India ink granules in histiocytes (experimental interval: 24 hours). Microphoto. Standard magnification.

* It is a question of the peculiar communication system in the surface layers of the membranes (stomata in in the mesothelium, "windows" of the marginal membrane and openings in the loops of the fibrous network), which are known in the literature as "hatches".

What happens to the various cellular elements (which constitute the mesothelial cells, the blood corpuscles, etc.) and their decomposition products, which are always present in the cavity of the testicular tunica vaginalis propria? There are two important facts which can be helpful in solving this problem: first, that, in the majority of mammals,* this cavity is connected with the peritoneal cavity by the uncovered processus vaginalis peritonei, and therefore, ways exist for the transfer of fluid and suspended particles from the serous cavity of the scrotum into the peritoneal cavity; and second, that the transfer of suspensions absorbed by the surface of the tunica vaginalis propria into the drainage system is possible by means of macrophage elements.

SUMMARY

Absorptive properties of the testicular tunica vaginalis propria are distinctly manifested only in case of true and colloidal solutions. Morphological experiments have clearly demonstrated that crystalloids are absorbed both by blood and lymphatic vessels of this serous membrane, while colloids only by its lymphatic vessels. In both cases the outstanding role is that of the vascular bed of the parietal sheath. The ability of the tunica vaginalis propria to resorb suspensions is insignificant or even nil.

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* Only in man, manlike apes and in some kinds of marsupials is the cavity of the testicular tunica vaginalis propria closed due to the obliteration of the processus vaginalis peritonei [4].

** In Russian.

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE IN ENGLISH TRANSLATION

May, 1957

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[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Sciences USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

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Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Eksptl. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimicheskii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimii	Uspekhi Biokhimii	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologii	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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